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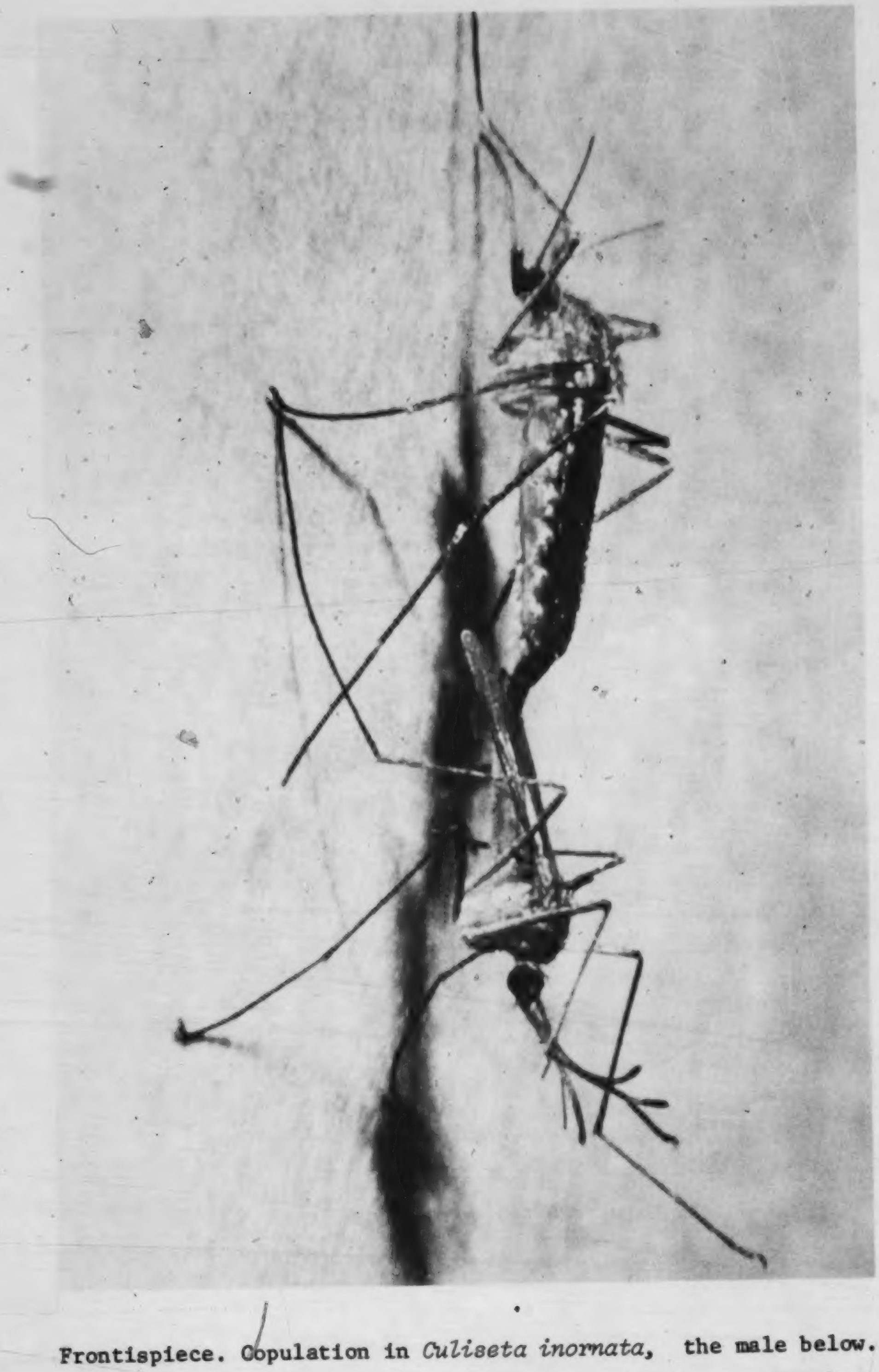

Frontispiece. Copulation in *Culiseta inornata*, the male below. (Photo by James K. Ryan).

THE UNIVERSITY OF ALBERTA

SEASONAL BIOLOGY OF *ANOPHELES*, *CULEX* AND *CULISETA* IN CENTRAL ALBERTA, (DIPTERA: CULICIDAE)

by

© James Edward Hudson

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

EDMONTON, ALBERTA

SPRING, 1977

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Seasonal biology of *Anopheles, Culex* and *Culiseta* in central Alberta, (Diptera: Culicidae).

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To the memory of

BRIAN HOCKING

ABSTRACT

Anopheles earlei Vargas, *Culex territans* Walker, *Culiseta alaskaensis* (Ludlow), *Culiseta inornata* (Williston), *Culiseta morsitans dyari* (Coquillett) and a few *Culex tarsalis* Coquillett, *Culiseta impatiens* (Walker) and *Culiseta silvestris minnesotae* Barr were studied at George Lake (53° 57' N, 114° 06' W) and Edmonton.

From May to October adults were collected mainly from New Jersey traps, windows and box shelters. Biting activity of *Cs. alaskaensis* and *Cs. inornata* was measured by collections from cattle, and of *Cs. m. dyari* by collections in quail-baited traps. Larvae were collected from a large permanent pond. Nulliparous *An. earlei*, *Clx. territans*, and *Cs. alaskaensis* appeared less than two weeks after snowmelt, but *Cs. inornata* not until about 7 weeks after snowmelt and most were gravid or parous. The even later appearance of female *Cs. m. dyari* and the collection of blood-feds, gravids and pars until October suggested that this subspecies overwintered in the egg stage. Females of all the other species were all unfed and nulliparous in September. Diapausing females (with undeveloped ovarian follicles) predominated by August 10 in *An. earlei*, (daylength 16:45 hr), August 5 in *Clx. territans* (16:57 hr) and August 18 in *Cs. inornata*, (16:00 hr); these three species were bivoltine. Females of the univoltine *Cs. alaskaensis* which emerged in June and July were all diapausing and none appeared at bait. No diapausing pars were seen in any species. Well-developed fatbodies and nectar-filled crops were seen not only in diapausing females in the fall but also in gonoactive and parous

females in summer.

Most diapausing *Cs. inornata* collected in September took small blood meals from the author in the laboratory, but did not digest them nor produce eggs. Follicles grew in some females held at 16:00 hours daylength and 20 C for 7 days, and such females did produce eggs after a blood meal. Very few wild-caught diapausing *An. earlei*, *Clx. territans* and *Cs. alaskaensis* took blood in the laboratory, and none produced eggs.

Life histories of *Cs. inornata* females in nature were reconstructed from laboratory measurements of rates of development at different temperatures, and records of air and pond water temperatures at George Lake. The first diapausing females would have experienced a decrease in daylength of 1:49 - 2:00 hours and a decrease in temperature of 6.8 - 7.2 C between hatching and eclosion. Diapause in laboratory-reared *Cs. inornata* was induced by a one-step decrease in daylength from 16:00 to 12:00 hours at the fourth larval instar or at pupation, but not by rearing at 16:00 or 12:00 hours throughout.

Female *An. earlei* spent the winter (November - March) in rockpiles, badger burrows and root cellars, and *Clx. territans* in rockpiles. Ten-day mean temperatures in winter were -3 to -13 C in rockpiles, -1 to -5 C in a burrow, +3 to -23 C in the outside air and always above 0 C in root cellars. Supercooling points of *An. earlei* and *Clx. territans* from the rockpiles suggested that they spent most

of the winter supercooled. Median lethal times of wild-caught females at -5 C were 137 days for *Clx. territans* but only 20 days for *Cs. inornata*. All the *An. earlei* and *Clx. territans* collected in winter were unfed nullipars.

It is concluded that none of the species is likely to overwinter arboviruses, because all the females collected in fall and winter were nulliparous, and none took blood.

PREFACE

I was born in Salisbury, England, in 1940, studied at St. Christopher School, Letchworth, and the University of Sheffield, where I graduated with a B.Sc. degree in zoology in 1963.

I became interested in mosquitoes while testing insecticides against them at the Tropical Pesticides Research Institute, Arusha, Tanzania, from 1966-70. I came to Canada in 1971 for graduate studies in Entomology, under the late Dr. B. Hocking.

My interest in Western Encephalitis began when I prepared a seminar on the ecology of the virus for a course in Medical and Veterinary Entomology (Ent. 492). The papers of McLintock et al. (1970) on the ecology of WE in Saskatchewan and of Shemanchuk (1965) on the overwintering of adult mosquitoes were major influences on my choice of thesis topic.

ACKNOWLEDGEMENTS

Mr. P. Addison, Department of Botany, gave advice on recording temperatures and adapted one of his computer programs for me. Messrs M. McIntyre and B. M. Rolseth, Department of Entomology, assisted me with techniques and construction of apparatus. Dr. J. D. Edman and Mrs. H. Lynn, Florida Medical Entomology Laboratory, Vero Beach, identified blood meals and Dr. O. Morgante, Provincial Laboratory of Public Health, Edmonton, conducted tests for viruses. Temporary assistance in the laboratory and in the field was provided by Mr. B. Boisvert, Mr. K. M. Boyd, Miss J. Marr and Miss J. Copeland. About 20 University of Alberta students and staff kindly came out in winter to help search rockpiles for mosquitoes. Computek, Ltd, were hired to help with computer analysis of the dissection records.

Mr. E. Donald, the farmer at George Lake, and his family provided bait cattle, and helped in many other ways.

My Supervisor, Dr. R. H. Gooding, my former Supervisor, the late Dr. B. Hocking, and other members of my committee gave help and encouragement of many kinds. Discussions and correspondence with Dr. R. E. Bellamy, Agriculture Canada, Saskatoon and Dr. R. A. Brust, University of Manitoba, Winnipeg were especially helpful in planning the study.

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1. INTRODUCTION

1.1. Western Encephalitis in Canada

Western encephalitis (WE, western equine encephalomyelitis, WEE, sleeping sickness) is a disease caused by a group A arbovirus, often fatal in equines and sometimes in humans. It occurs mainly in western North America and *Culex tarsalis* is the principal epizootic and enzootic vector, (James and Harwood, 1969). In Canada the most severe recorded epizootics have been in Saskatchewan; in 1938 there were 15,000 deaths and an estimated 50,000 cases in horses, (Dillenberg, 1965). In Alberta in 1965 there were 322 reported and 63 confirmed horse cases, (Morgante, Shemanchuk, Vance and Windsor, 1968a), 1 - 3 reported cases each year in 1966 - 74, and 120 reported and 19 confirmed cases in 1975, (Dr. R. G. Christian, Alberta Veterinary Services Laboratory, Edmonton, personal communication). Confirmed human cases have been recorded in Alberta from Lethbridge, Medicine Hat and Hanna (Morgante, Barager and Herbert, 1968b), and 20 of 160 (12 %) residents of Rochester (54° N) had neutralising antibodies for WE, (Iversen, Seawright and Hanson, 1971). Human and horse cases are also known from British Columbia and Manitoba, (review by McLintock and Iversen, 1975).

The reservoir hosts and distribution of WE in northern Canada are little known. In Saskatchewan the virus has been isolated from english house sparrow (*Passer domesticus*), mourning dove (*Zenaidura macroura*), Swainson's hawk (*Buteo swainsoni*) and Richardson's ground squirrel (*Spermophilus richardsoni*), and neutralising antibodies have been found in another 9 species of wild birds and 4 of wild mammals, (Burton et al, 1966; Burton and McLintock, 1970). Neutralising antibodies and virus isolations from garter snakes and leopard frogs have been reported (Burton, McLintock and Rempel, 1966), but the virus isolation techniques used were unreliable (Reeves, 1974). The virus may be introduced to new areas by migratory birds such as Swainson's hawk, which nests in the Northwest Territories, (Godfrey, 1966). This may be the means by which the virus was introduced to Atkinson Point (69° 30' N), where WE antibodies were detected in reindeer, (Burton and McLintock, 1970).

WE virus has been isolated from *Culex tarsalis*, *Culiseta inornata*, *Aedes campestris*, *Aedes dorsalis*, *Aedes flavescens*, *Aedes spencerii* and *Aedes vexans* in Saskatchewan (McLintock et al, 1970). Most isolations were from *Clx. tarsalis*, and most were from the prairie and aspen grove zones in the southern part of the province. The most northerly isolations were from *Cs. inornata* at St. Walburg (53° 39' N) in the boreal forest zone, where this species was common in light traps, but *Clx. tarsalis* was rare. The authors concluded that *Cs. inornata* might be an important transmitter of WE to horses in Saskatchewan, because of the frequency of virus isolations, its abundance, and its apparent preference for feeding on larger mammals. In Alberta WE virus isolations have been made from *Clx. tarsalis*, *Cs. inornata* and *Ae. vexans* in the irrigated prairie regions (Shemanchuk and Morgante, 1968), and the most northerly isolation has been from *Clx. tarsalis* at Stettler (52° 10' N) in the aspen grove zone (Hall, McKiel and Brown, 1968).

Of the 63 confirmed cases of WE in horses in Alberta in 1965, 21 were from Edmonton and further north and the most northerly was from Peace River (56° 10' N), (Morgante et al, 1968a). *Culex tarsalis* adults have been taken at Norman Wells, N.W.T., (Jenkins, 1950), but the most northerly record from Alberta is from Lac La Biche (54° 40' N), (J. H. Brown, unpublished report, 1967). In two large collections north of Edmonton, *Clx. tarsalis* was rare at George Lake (53° 57' N), (Graham, 1969b) and absent from Flatbush (54° 40' N), (Happold, 1965a). *Culiseta inornata*, however, is common at Edmonton and George Lake and has been recorded at Deadwood (W. Inkpen, Alberta Agriculture, unpublished report, 1973) and Fort McMurray (J. A. Shemanchuk, Agriculture Canada, unpublished data, 1973), both sites at around 56° 40' N, in the boreal forest zone (LaRoi, 1968). Thus on distributional grounds *Cs. inornata* deserves further consideration as a vector in central and northern Alberta.

In Saskatchewan in 1963 the peak number of horse cases of WE was in mid-August, about 2 weeks after the peak numbers of *Culex* and *Culiseta* in traps and the peak of WE virus isolations from mosquitoes, (McLintock et al, 1966). In Alberta in 1965 the peak number of confirmed horse cases was in the fourth week in August (Morgante et al, 1968a). Mosquito data for the same year are not available, but in an earlier survey near Brooks, (Shemanchuk, 1959a) peak numbers of *Clx. tarsalis* and *Cs. inornata* were trapped in the second week of August. The earliest virus isolation in Saskatchewan was from a Richardson's ground squirrel on May 19 (Burton et al, 1966) but the first virus isolations from mosquitoes were not until June

16 - 18 (*Aedes campestris*) and June 22 (*Culex tarsalis*), McLintock et al, 1970). At Rochester only 2 % of the adult snowshoe hares had antibodies to WE in April but 97 % had them in June, 1963 (Yuill and Hanson, 1964). There were no isolations of WE virus from mosquitoes during these months, (Iversen et al, 1969). In studies in southern Alberta, indicator chickens did not become seropositive until 27 July - 10 August, though they had been exposed since May (Morgante, Shemanchuk and Windsor, 1969), and WE virus was isolated from mosquitoes only in August (Shemanchuk and Morgante, 1968).

Thus most of the recorded human and horse cases have been after the apparent population peaks of *Clx. tarsalis*, but virus activity in wild mammals and birds has been detected before any virus isolations from mosquitoes. Seropositive humans and confirmed horse cases have been recorded from the boreal forest zone, where *Clx. tarsalis* seems rare but *Cs. inornata* is common. These findings indicate the need for further studies of mosquitoes in the early spring, particularly those which overwinter in the adult stage, and they indicate that *Cs. inornata* may be the primary vector at least to horses, in the boreal forest zone.

1.2. Mosquito life cycles in the northern temperate regions

Life cycles may be divided into the following groups, according to the scheme originally devised by Wesenberg-Lund (1921) for the Danish mosquitoes, and modified by Bates (1949) and Frohne (1954b). The local examples are taken from Rempel (1953) unless otherwise indicated.

I. Overwintering is in the egg stage with only one generation per year. This group, the "univoltine *Aedes*" (e.g. *Ae. communis*, *Ae. fitchii*) includes most of the *Aedes* found in Alberta, and *Culiseta morsitans dyari* (Morris, Zimmerman and Magnarelli, 1976).

II. Overwintering in the egg stage and sometimes several generations per year. This group, the "multivoltine *Aedes*" has fewer species in Alberta but it includes two serious pests, *Ae. dorsalis* and *Ae. vexans*.

III. Overwintering in the larval stage with usually only one generation per year. This is the "*Anopheles claviger* type of cycle" of Bates (1949), and may be represented in Alberta by *Coquillettidia perturbans* (= *Mansonia*), though there have been no local investigations to confirm this.

IV. Adult females overwinter and there may be several generations per year. My own observations suggest that *Anopheles earlei*, *Culex territans* and *Culiseta inornata* show this type of life cycle in central Alberta.

V. The adult females overwinter, and there is only one generation per year, because the females that emerge in summer do not take blood until the next spring. This is the "*Culiseta impatiens* life cycle" of Frohne (1954b), and *Cs. alaskaensis* also displays it.

1.3. Overwintering of arboviruses

Reeves (1974) reviewed hypotheses based on virus persistence through:

> "a long-lived and persistently infected primary vector, an infected primary vector capable of transovarian transmission of virus to progeny, alternative arthropod or metazoal vectors, chronic latent infection in vertebrate hosts, annual reintroduction of infection by infected migratory or dispersing hosts or vectors, or persistence in food chains of primary vectors and hosts."

The author concluded that the reservoiring process for most arboviruses was poorly understood, and that it would not be surprising if arboviruses had alternative means to survive adverse periods. WE virus persisted in experimentally infected *Culex tarsalis* from October to May under outdoor conditions in California (Bellamy, Reeves and Scrivani, 1967), but no virus was isolated from wild-caught *Clx. tarsalis* for a two-month period in mid-winter. One isolation of WE virus was made from *Clx. tarsalis* overwintering in mines in Colorado, on 31 December, but 30 other pools were negative, (Blackmore and Winn, 1956). In Oregon, the virus was isolated from *Clx. tarsalis* in two summers, but not in the winter between, (Rush, Kennedy and Eklund, 1963). Transovarial overwintering of La Crosse virus in *Aedes triseriatus* has been found in nature in Wisconsin, (Watts et al, 1974) but several studies of transovarian transmission of WE virus have given only negative results (references in Reeves, 1974).

Of the other hypotheses offered to explain overwintering of arboviruses, Reeves (1974) considered that the most attractive involved latent infection in hibernating vertebrate hosts. Richardson's ground squirrel may be a winter reservoir and an early spring amplifier host of WE in Saskatchewan (Leung et al, 1975).

Another species of ground squirrel (*Spermophilus lateralis*) experimentally infected with Coxsackie B-3 virus showed latent infection during hibernation, followed by viremia on arousal, (Dempster, Grodums and Spencer, 1966). Adult mosquitoes uninfected in fall and winter could still become infected early in spring by feeding on vertebrates with recrudescent viremia.

1.4. Overwintering of adult mosquitoes in Alberta

Investigation of the role of mosquitoes as winter reservoirs of WE in the northern part of its range have been hampered by difficulties in finding them. Studies of the overwintering of *Clx. tarsalis* in the northwestern United States, (e.g. Rush, 1962) have yielded some records for other species (see Chapter 7). In the only previous study in Alberta, Shemanchuk (1965) trapped *Anopheles earlei*, *Culex tarsalis* and *Culiseta inornata* entering mammal burrows in fall and leaving them in spring. WE virus isolations were made from females in the same burrows up to August 19 (*Cs. inornata*) and August 25 (*Clx. tarsalis*) but not in September (Shemanchuk and Morgante, 1968). Since females continued to enter the burrows until November it is not certain that the infected females were the ones which overwintered. No virus isolation attempts were reported from the females in winter or spring.

1.5. Reproductive diapause in female mosquitoes

In most mosquitoes that overwinter, the eggs, larvae or females, depending on species, undergo diapause, defined as "a genetically induced state of arrested development, the manifestation of which may be induced by environmental factors", (Beck, 1968). Diapause in mosquitoes has been reviewed by Clements (1963) and Vinogradova (1969).

Diapausing females do not produce eggs, and most species do not take blood meals, but a few do. The ovaries remain small and the fatbody is often well-developed. Diapausing females may be quite active, feeding on carbohydrate sources such as nectar, and sometimes fly a long way from breeding to overwintering sites.

The only known way that female mosquitoes could overwinter WE virus would be to feed on viremic hosts before or during winter. If egg production always follows a blood meal, the absence of blood-fed, gravid or parous females from collections in winter rules out the possibility of virus infection. Females that have laid eggs before may be recognised by the uncoiling of the tracheoles on the ovaries (Detinova, 1962). However, females of some species feed on blood in winter without maturing eggs, a phenomenon known as gonotrophic dissociation, and so may become infected without leaving a record in the ovary. This phenomenon has haunted workers on the overwintering of diseases ever since its discovery in the early days of malariology.

In investigations in the Netherlands, Swellengrebel (1929) noted that *Anopheles labranchiae atroparvus* bit humans in houses in fall as they had in summer, but instead of maturing eggs and leaving the houses to lay them, the mosquitoes remained in the houses and continued to take blood meals, but produced no eggs until late winter or spring. This increase in repeated feeding on humans caused fall epidemics of malaria. The mosquitoes alone could not overwinter the malaria parasites, however, because oocysts and sporozoites degenerated about 50 days after infection, (Hackett, 1937).

Swellengrebel (1929) defines gonotrophic dissociation as follows, (my translation from French): "In spite of the halt in egg production (reproductive activity) the *Anopheles* continue to nourish themselves on blood (nutritive activity)". A later paper (deBuck and Swellengrebel, 1934) demonstrated that *An. l. atroparvus* needs blood in winter to survive. Only 5 % of the females survived the winter in a cool outhouse, but 59 % of the closely-related *An. maculipennis messeae* survived under the same conditions. *An. m. messeae* had much better developed fatbodies at the beginning of winter, hibernated in outside shelters and did not take blood meals. This was described as "gonotrophic concordancy" (no feeding - no eggs). The authors intended that the two terms be used only to describe what the mosquitoes do under natural conditions, but this restriction has not been observed by some later workers, such as Eldridge (1968).

Gonotrophic dissociation has been seen under natural conditions in *An. m. messeae* near Leningrad in August (Danilevskii and Glinyanaya, 1958), and also near Omsk in January (Zhukov and Krasikova, 1942), in *An. m. typicus* and *An. l. atroparvus* near Belgrade (Guelmino, 1951), in *An. sacharovi* (= *elutus*) in Israel (Mer, 1931), and *An. freeborni* in California (Freeborn, 1932, Washino, 1970). All these are members of the *Anopheles maculipennis* complex. Buttiker (1958) found blood-fed *An. culifacies* in Ceylon and Burma showing no egg development, though the blood meals were dark and almost dry. There are few observations of gonotrophic dissociation in Culicines under natural conditions. Oda and Wada (1973) found that *Culex tritaeniorhynchus summorosus* in pigsties near Nagasaki had a gonotrophic dissociation rate of 1.3 % in March - May, 6.0 % in June - August, and 12.4 % in September - October, but the feeding rate decreased so much in fall that the authors conclude that the gonotrophic dissociation was of no epidemiological importance. Shemanchuk and Morgante (1966) report that 50.1 % of the *Culiseta inornata* and 8.7 % of the *Culex tarsalis* they collected in burrows from 4 August to 15 September were blood-fed, and the remainder were unfed but fat. There was no sign of egg development although some of the meals were partly digested. This was interpreted by the authors as gonotrophic dissociation.

Daylength (photoperiod, the number of hours of light per day), has been shown to be critical in the induction of diapause in many insect species; the subject is reviewed by de Wilde (1962), Danilevskii (1965), Beck (1968) and Tauber and Tauber (1973a). Several laboratory studies have demonstrated that daylength and temperature affect blood feeding and egg development in mosquitoes. In *An. m. messeae*, *An. l. atroparvus*, *An. hyrcanus* and *Culex pipiens*, females reared under short days failed to mature eggs after a blood meal, (Vinogradova, 1960). In *An. punctipennis* and *An. freeborni* fewer females took blood meals when reared under short than long days, (Washino, 1970; Washino and Bailey, 1970). In *An. freeborni* the short-day, blood-fed females showed gonotrophic dissociation, and developed as much fat as sugar-fed females, (Washino, Gieke and Schaefer, 1971).

Fewer females took blood and fewer blood-feds matured eggs under short than under long days, in *Culex pipiens* (Eldridge, 1968), and *Clx. restuans*, (Eldridge, Bailey and Johnson, 1972). In another study in *Culex pipiens*, (Sanburg and Larsen, 1973) and one on *Culiseta inornata* (Kalpage, 1970), rearing under short days reduced the blood feeding rates, but in both studies most of the blood-fed females matured eggs and thus were not in diapause according to Vinogradova's criterion (1960). Blood feeding under laboratory conditions may be a poor indicator of what will happen in nature, because several steps of the normal host-finding process (Hocking, 1971) are usually omitted. If the diapausing females do not take blood in nature it is better to measure the onset of diapause by other means. Another difficulty is that if wild-caught mosquitoes are experimentally fed and mature eggs, it can not easily be determined if they were parous at the time of feeding.

In members of the *Culex pipiens* complex (Oda, 1968; Spielman and Wong, 1973a) and in *Culex tritaeniorhynchus* (Kawai, 1969), females in late summer and fall have small, undeveloped ovarian follicles. Laboratory studies on *Culex pipiens* (Oda, 1971; Sanburg and Larsen, 1973; Spielman and Wong, 1973b), *Culex tritaeniorhynchus* (Kawai, 1969), *Culex tarsalis* (Harwood and Halfhill, 1964), and *Anopheles freeborni* (Depner and Harwood, 1966) showed short days restricted ovary or follicle length.

Two drawbacks in using follicle development as an indicator of diapause are that this character can only be seen on dissection, and that a newly emerged female whose follicles are still growing can be mistaken for one in diapause. The changes that take place in the abdomen of a female mosquito, exemplified by *Culiseta inornata* from the time she emerges are shown in Fig. 1. Teneral (newly-emerged) individuals are recognisable by larval muscle remnants, but these are autolysed in 2 1/2 days at 15 C (see Chapter 5). At emergence the primary ovarian follicles are not yet formed, but by the end of the teneral stage the follicles have budded from the germaria and the oocyte may have begun to differentiate. (Terminology is explained in Chapter 2 and Detinova, 1962). In a diapausing female the follicles are arrested at an early stage of differentiation, and the female increases her fatbody reserves by feeding on nectar, and possibly blood. In a non-diapausing (gonoactive) female the follicles grow and there is usually some yolk deposited in the oocytes before she takes her first blood meal. It is not known how long after the end of the teneral stage a non-diapausing female takes her first blood meal; it may be 2 days in summer. Nor is it known exactly when diapause ends in winter, and under outdoor conditions the cold will keep the female quiescent until spring. The gonoactive nullipar's first blood meal results in egg production. After the eggs are laid the parous female may complete several more gonotrophic cycles. Pars are not known to diapause.

In California overwintering *An. freeborni* reach their maximum lipid content in October, but in *Clx. tarsalis* this does not

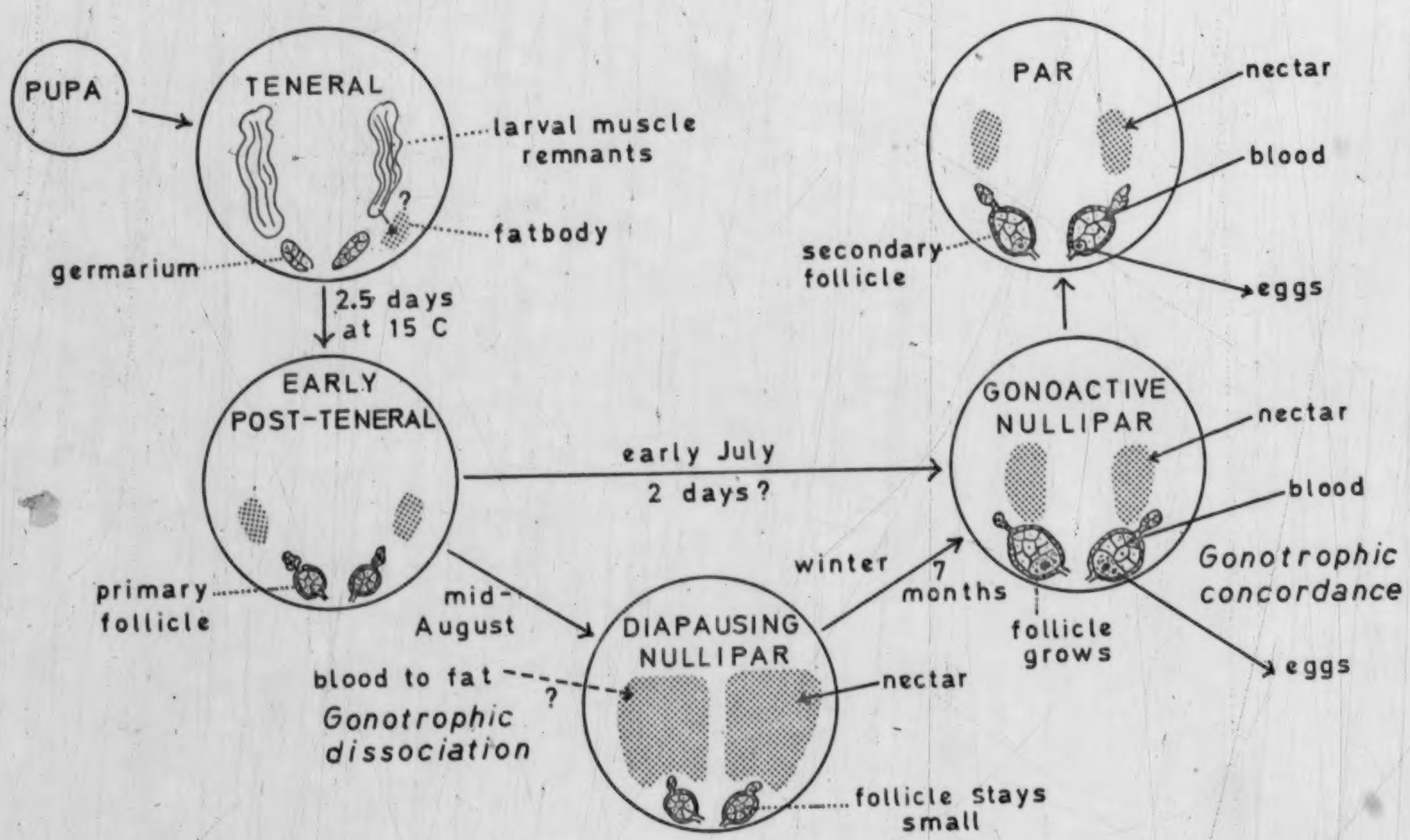

PUPA
TENERAL
larval muscle remnants
?
fatbody
germarium
2.5 days at 15 C
EARLY POST-TENERAL
primary follicle
early July
2 days?
mid-August
DIAPAUSING NULLIPAR
blood to fat
?
Gonotrophic dissociation
nectar
follicle stays small
winter
7 months
GONOACTIVE NULLIPAR
nectar
blood
Gonotrophic concordance
follicle grows
eggs
PAR
nectar
blood
secondary follicle
eggs

Fig. 1. The teneral stage and reproductive diapause in the female mosquito, exemplified by *Culiseta inornata.*

occur until November (Schaefer, Miura and Washino, 1971). Females of both species in the laboratory develop much bigger fatbodies on a sugar diet when kept under short than under long days, (Depner and Harwood, 1966; Harwood and Halfhill, 1964). According to Wallis, (1959) laboratory-reared *Culex restuans* prefer sugar to blood in late summer. During fall and winter in California *Clx. tarsalis* feed on sugars which they convert to triglycerides (Schaefer and Miura, 1972). Roubaud (1932) suggested that in *Culex pipiens* the products of autolysis of the larval muscles were the major source of winter food reserve. Clements (1956) noted that while the fatbody was quite well developed in *Clx. pipiens* at adult emergence, it was still better developed 3 days later, after the larval muscle remnants had gone. In another study with *Clx. pipiens* Tekle (1960) found that newly-emerged females contained about 20 % fat, increasing to 35 % after 2 weeks on sucrose solution. According to Maslov, (as described by Vinogradova, 1969), newly-emerged, unfed female *Culiseta alaskaensis*, *Culiseta bergrothi* and *Allotheobaldia longiariolata* have better developed fatbodies if the larvae are reared at low rather than high temperatures and under short rather than long days. Both male and female mosquitoes take nectar from flowers (Grimstad and DeFoliart, 1974; and review by Hocking, 1953), but it is not known how much of the female's winter food reserve is accumulated in the larval and how much in the adult stage. Wild-caught *Culex tarsalis* caged outdoors in the fall grew fat when provided with 10 % sucrose but soon died without it, (Bennington, Sooter and Baer, 1958).

Prehibernation dispersal flights have been studied in *Anopheles sacharovi* (Kligler and Mer, 1930), *An. freeborni* (Rosenstiel 1947; and others), and *Culex tarsalis* (Kliewer, Miura and Chapman, 1969). *An. sacharovi* moved 14 km and *An. freeborni* 42 km from their breeding sites and both took blood meals on the way.

With adult mosquitoes, as with most insects, (Tauber and Tauber, 1976) there has been far less study of the factors that maintain and terminate diapause than of those that induce it. In *Culex pipiens* diapause is terminated fairly rapidly by long days (Sanburg and Larsen, 1973; Spielman and Wong, 1973b; Oda and Kuhlow, 1974), or continuous illumination (Tate and Vincent, 1936). In October *Anopheles m. messeae* showed anomolous blood digestion while *An. l. atroparvus* showed gonotrophic dissociation, but by January both subspecies showed normal blood digestion and egg production (de Buck and Swellengrebel, 1934). Before *Culex pipiens pallens* and *C. p. pipens* leave their overwintering sites their ovarian follicles have grown to the size and stage normally associated with gonoactive females (Oda, 1968; Oda and Kuhlow, 1973), but in *C. p. pipiens* the blood feeding rate of the supposedly gonoactive females in April was still very low, (Oda and Kuhlow, 1974). *Culiseta annulata* collected from hibernation in England and fed blood experimentally, produced eggs if the temperature after feeding was 13.5 C or above and increased their fatbody if it was 10.4 C or below. Daylength seemed to have no influence and the same females could be made to produce either fat reserves or eggs after alternate feeding, (Service, 1968b). This is a most interesting form of gonotrophic dissociation in that it is facultative and seems to be independent of daylength, though daylength might have played its part in inducing diapause in the females before they were collected. Ways of measuring the duration and intensity of diapause are of epidemiological interest because of the possibilities of feeding on vertebrates in mutual overwintering sites.

The appearance in August of gonotrophic dissociation in *Anopheles m. messeae*, (Danilevskii and Glinyanaya, 1958), of *Culex pipiens* with diapause follicles, (Spielman and Wong, 1973a), and the early cessation of blood feeding in *Culex tarsalis*, (Bennington, Sooter and Baer, 1958), suggest that in these species the cessation of reproduction is not a direct response to winter cold, or "quiescence" in Shelford's terminology (Lees, 1955). Females showing gonotrophic dissociation under laboratory conditions have been described as being in "imaginal diapause" (Vinogradova, 1960), and females with undeveloped ovarian follicles as "gonoinactive" (Oda, 1968) or in "ovarian diapause" (Spielman and Wong, 1973b). I prefer the term "reproductive diapause" to "ovarian diapause", since the condition may affect more than the ovary. Later in this thesis, post-teneral females will be classed as diapausing or non-diapausing according to their follicle: germarium ratios; the critical value for each species was determined by field observations on the time of cessation of blood-feeding in the population. In gonoactive, anautogenous females before the first blood meal the ovarian follicles stop growing at a "primary resting stage" (Rosay, 1969). This varies with species, but in some species at least the resting stage follicles of gonoactive females are considerably larger than those of diapausing females.

1.6. Objectives of the study.

The aim was to study the biology of *Anopheles*, *Culex* and *Culiseta* species throughout the year, with emphasis on those features that would enable them to overwinter WE virus. The following specific topics were investigated.

a) The number of generations per year;

b) The time of onset of diapause;

c) The blood feeding and sugar feeding habits of females;

d) Seasonal fatbody development;

e) Blood feeding and assimilation in diapausing females;

f) Laboratory induction of diapause;

g) The overwintering sites of the females; and

h) Cold-hardiness of the females.

This broad approach was dictated by not knowing beforehand if the females could be found during winter. If they could not, some idea of their epidemiological importance might be gained from their feeding habits in summer and fall.

1.7. Review of the literature on *Anopheles*, *Culex* and *Culiseta* of Alberta

Since Pucat's (1965) list of mosquito records from Alberta, Graham (1969) added *Culiseta silvestris minnesotae*, and there are four more *Aedes* records, *Ae. abserratus* (Ellis, personal communication), *Ae. churchillensis*, (Ellis and Brust, 1973), *Ae. barri* and *Ae. mercurator* (Enfield, 1976). The record for *Ae. niphadopsis* is probably invalid since the only specimen so named in our collection is an *Ae. dorsalis* (Ellis, in litt.). This brings the total to 41 species: 30 *Aedes*, 1 *Anopheles*, 1 *Coquillettidia*, 3 *Culex* and 6 *Culiseta*. The nomenclature here and elsewhere in this thesis follows Stone, Knight and Starcke (1959) and supplement III (Stone, 1967).

The following notes on distribution, biology and medical importance do not give a complete list of references to any species and some papers mentioned elsewhere in this thesis will not be mentioned here. Distribution records are from Carpenter and LaCasse (1955) unless otherwise stated. References to overwintering sites are given in Chapter 7.

Anopheles (*Anopheles*) *earlei* Vargas 1943

This recently described member of the *Anopheles maculipennis* complex is distributed across the northern United States and Canada and any early records for *An. maculipennis* in this region probably refer to *An. earlei*. Kitzmiller, Frizzi and Baker (1967) consider the Nearctic species of the *An. maculipennis* complex are a less closely related group than the Palearctic species; crossing experiments suggest that *An. earlei* is closest to *An. punctipennis*. Rozeboom (1952a) noted that the eggs of *An. earlei* and the Palearctic *An. maculipennis typicus* were very similar to each other but quite different from those of other members of the complex.

The adult females overwinter and in Alaska there is only one generation per year (Frohne, 1954b). The larvae are found in permanent pools, swamps and the edges of lakes, often with abundant floating or emergent vegetation, (Hearle, 1926; Jenkins, 1948; Irwin, 1942), and also at the edges of streams (McLintock, 1944).

There is no direct evidence that *An. earlei* is a malaria vector, but on the basis of its present distribution O'Rourke (1959) considered that it may have been a vector in Ontario in the 19th century. The females are efficient vectors of dog heartworm (*Dirofilaria immitis*) in the laboratory, (Yen, 1938). Although there are records of attacks on man, the species is not considered a severe pest. Laboratory colonization is reported (Rozeboom, 1952b; Kreutzer and Kitzmiller, 1969). The biology of *An. earlei* is also reviewed by Barr (1958).

Culex (*Culex*) *restuans* Theobald, 1901

This species is widely distributed in North America, and has been found infected with WE virus in Manitoba (review by McLintock and Iversen, 1975), I did not find any specimens in the present study.

Culex (*Culex*) *tarsalis* Coquillett, 1896

This Nearctic species is commonest in the western, central and southern United States and southwestern Canada, but there is a record for the Northwest Territories (see Section 1.1.). Because of its importance as the vector of WE, (Section 1.1.), this species has been much studied and only a few of the references are given here.

Variations in the life cycle over the range of *Clx. tarsalis* are reviewed by Nelson (1971). The adult females are gonoinactive for 8 months in Canada, but only 2 - 3 months in central California, and in southern California breeding occurs all the year round. Larvae are found in a wide variety of clear and polluted waters, (Carpenter and LaCasse, 1955).

The importance of *Clx. tarsalis* in the ecology of WE seems to be due to its high infectivity (Thomas, 1963) and to its feeding both on mammals and on birds. Shifts in the population from feeding mainly on birds in spring to mainly on mammals in summer have been reported (Tempelis et al, 1967).

Culex (*Neoculex*) *territans* Walker, 1856

This Holoarctic species is found throughout Canada and the continental United States, including Alaska, (Carpenter and LaCasse, 1955), Europe, North Africa, Asia Minor and Japan (Gutsevich, Monchadskii and Shtakel'berg, 1974). Records of *Clx. apicalis* from east of the Rocky Mountains probably refer to *Clx. territans*.

The females overwinter and there is only one generation per year in Alaska (Frohne, 1954b), but there may be several, or continuous development, in the southern part of the range (Horsfall, 1955). The larvae have usually been found in permanent pools with floating or emergent vegetation (Jenkins, 1948; and others), also in roadside ditches (Hearle, 1926), rain barrels, (Thibault, 1910), and rock pools (Natvig, 1948). In a Virginia wood females showed the greatest flight activity around dawn and dusk, with some during daylight (Gladney and Turner, 1970). Diurnal swarming of males has been observed in Alaska (Frohne, 1954c).

In the northeastern United States blood meal identifications indicated that most *Clx. territans* females had fed on amphibians, with a few bird, mammal, and reptile feeds, and feeding on frogs was observed in nature, (Crans, 1970). In field tests with paired bait animals, almost equal numbers came to reptiles and amphibians, very few to birds and none to mammals, (Means, 1968). A female was seen attacking man in nature (Means, 1965). In Winnipeg, however, McLintock (1944) described it as "probably the most important mosquito pest in the area", and Horsfall (1936) described *Culex territans* in Arkansas as "of general economic importance" and "very annoying". It seems likely that these two authors were writing about a different species.

Eastern encephalitis (EE) virus has been isolated from *Clx. territans* in New York, (Morris et al, 1973), and this mosquito has been shown to transmit the filarioid worm *Foleyella flexicana* to bullfrogs in the laboratory, (Benach and Crans, 1973). Laboratory colonies have been described (Benach, 1970; Chapman and Barr, 1969).

Culiseta (*Culiseta*) *alaskaensis* (Ludlow, 1906)

This Holarctic species occurs in the tundra and taiga regions of North America and Europe, and in the Rocky Mountains as far south as Colorado.

Frohne (1954a) made a detailed study of *Cs. alaskaensis* in southern Alaska. The adult females emerge from their overwintering sites at snowmelt and take blood, and there is only one generation in the season. The females have an obligatory diapause. In the laboratory they will not take blood until 3 months after emergence and in nature they do not take blood until spring of the next year.

Larvae are found mostly from May to August, in *Carex* marshes, duckweed ponds, coastal marshes (Jenkins, 1948), rock pools (Hocking, Richards and Twinn, 1950), and spruce forest pools (Curtis, 1953).

The females attack humans in early spring, during the daylight hours, at temperatures as low as 7 C (Jenkins, 1948) and winds as high as 24 - 32 km/hr, (Jenkins and Knight, 1950). Attack rates of 200 - 300 females per minute have been recorded on man and reindeer in forest tundra in the U.S.S.R. (Gomoyunova, 1973), and the species is a pest to humans in Alaska in spring (Frohne, 1954a). Northway virus (Bunyamwera Group) has been isolated from females in Alaska (review by McLean, 1975). Some data on development and feeding in the laboratory are given by Frohne (1954a); he did not establish a self-perpetuating colony because the adults did not mate. Male swarming has been observed in nature (Sommerman, 1964).

Culiseta (*Culiseta*) *impatiens* (Walker, 1856)

This Nearctic species is found across Canada and the United States, as far south as Missouri.

Frohne (1953) studied *Cs. impatiens* in the field and in the laboratory, and reviewed the literature on it. The adults mated readily and lived long in small cages, but the females did not bite readily or produce eggs until 3 - 4 months after emergence. The life cycle and larval habitats are similar to those of *Cs. alaskaensis*, except that the larvae are also found in roadside ditches, (Jenkins, 1948).

Cs. impatiens females were troublesome to humans during May and June in Alaska, and to cattle in late March in British

Columbia (Hearle, 1926).

Culiseta (Culiseta) incidens (Thomson, 1868)

This Nearctic species is found in western North America from southern California to Alaska, and has been recorded from Alberta but I have not found any specimens. The females are severe pests in some areas, and have transmitted Western, St. Louis and Japanese B Encephalitis viruses in the laboratory, (references in Carpenter and LaCasse, 1955).

Culiseta (Culiseta) inornata (Williston, 1893)

This Nearctic species has been found in all the continental United States except North Carolina and Alaska, in northern Mexico and in Canada as far east as Ontario; it seems to be more common in the west than the east. The biology of *Cs. inornata* has been reviewed by Barr (1958) and Washino et al (1962).

There may be several generations per year. In the north the females overwinter and larvae are found in the summer months. In the south the larvae are most common in winter and aestivation occurs, possibly in the egg stage (Buxton and Breland, 1952). Some data on seasonal occurrence of larvae and adults at different latitudes are summarised in Table 1. In southern Illinois (around 38° N) in the transition between the summer breeding and winter breeding parts of the range, there appear to be two peaks of adult activity, in April - May and September - October, (Ross, quoted by Barr, 1958). The shift in breeding season may be connected with the lower temperature optimum for development of *Cs. inornata* compared with

Table 1. Seasonal occurrence of larvae and activity of adults of *Culiseta inornata* at various latitudes.

Province or State	Approximate Latitude (N)	Adults			Larvae and Pupae			Author
		First	Peak	Last	First	Peak	Last	
Alberta	54	May	Aug	Oct	June	Aug	Oct	This report (section 3.12)
Alberta	50	May	Aug	Sept	May	July	Sept	Shemanchuk (1959a)
Manitoba	50	May	-	Sept	May	-	Sept	McLintock (1944)
Saskatchewan	50	May	-	Oct	May	-	Oct	Stewart (1973)
Washington	47	May	July.	Sept	May	-	Oct	Yates (1953)
Montana	47	June	-	Sept	June	-	Aug	Mail (1934)
Minnesota	45	April	-	Oct	-	-	-	Barr (1958)
Nebraska	42	-	-	-	April	July	Nov	Edmunds (1958)
Nebraska	41	March	-	-	April	-	-	Thompson (1953)
Utah	41	May	July	Oct	April	June	Sept	Collett et al. (1964)
California	38	-	-	-	Jan	-	May	Telford (1958)
Tennessee[1]	37	-	-	-	Aug	-	May	King et al. (1960)
California	35	Sept	May	July	Sept	Jan	July	Washino et al. (1962)
California	34	Sept	Dec	July	Oct	Jan	April	Apperson et al. (1974)
Arkansas	33	-	-	-	Dec	-	March	Horsfall (1937)
Louisiana	30	-	-	-	Dec	-	March	Dozier (1936)
Texas	26-36	-	-	-	Sept	-	March	McGregor and Eads (1943)

[1] From this line downwards, the "last month" is in the year after the "first month".

other species such as *Culex tarsalis*, (Rosay, 1973; Hanec and Brust, 1967). In Utah most pools with *Cs. inornata* larvae had 25 - 100 % shade and a mean temperature of 19.5 C while most pools with *Clx. tarsalis* and other species had less than 25 % shade and a mean temperature of 22.5 C, (Graham and Bradley, 1965). The larvae develop in a variety of clear, polluted and brackish waters. They are particularly abundant in waste irrigation waters, (Yates, 1953). Females fly 2 km regularly in search of blood meals (Owen, 1937), and at Chicago a marked female moved 22 km in 5 days, (Clarke, 1943). In a study with light traps in Georgia, females were taken throughout the night, with the greatest activity in the last quarter of the night, (Love, Platt and Goodwin, 1963). Copulation in nature occurs as soon as the female emerges, (Horsfall, 1955) and may last 3.5 - 6 hours (Rees and Onishi, 1951), though only 5 minutes is needed for sperm transfer (Kliewer et al, 1967). The male palp and antenna are much less hairy than in other local *Culiseta* and *Culex* species, (see frontispiece), and there is behavioural evidence that the female produces a sex pheromone (Kliewer et al, 1966).

Female *Cs. inornata* have transmitted the viruses of Western, St. Louis and Japanese B Encephalitis in the laboratory (review in Washino et al, 1962), and have been found infected with WE in nature in Washington, (Hammon et al, 1945), Alberta and Saskatchewan (see Section 1.1.). No virus was isolated however, from 4,900 females collected in an area of California where WE and SLE were enzootic (Washino et al, 1962). Moreover, females from Saskatchewan picked up and maintained a local strain of WE virus in the laboratory but did not transmit it by bite (Hayles, 1971). Blood meal identifications in several areas of North America revealed that almost all the *Cs. inornata* females had fed on large mammals, mostly cattle, (Tempelis, 1975). In Colorado, (Tempelis et al, 1967), Utah (Anderson, Collett and Winget, 1967), Washington (Reeves and Hammon, 1944) and southern Alberta (Shemanchuk, Downe and Burgess, 1963), *Culex tarsalis* were collected from the same areas as *Cs. inornata* and far more of the former had fed on birds. In southern Alberta, however, 308 of 1364 *Cs. inornata* females leaving chicken-baited traps (22.6 %) were blood-fed, (Shemanchuk, 1969).

Cs. inornata has also been found infected with California Encephalitis (CE) and Cache Valley viruses in Alberta and elsewhere (review by McLintock and Iversen, 1975), and with CE at Marsh Lake, Yukon (McLean, 1975). Marsh Lake CE virus persisted in intrathoracically inoculated Marsh Lake *Cs. inornata* females, for 77 days at 4.5 C followed by 117 days at -1 C, (McLean et al, 1975).

Continuous laboratory culture of *Cs. inornata* is quite easy at temperatures of 15 - 21 C; methods are given by Owen (1942), McLintock (1952, 1964), and Pappas, (1973). There have been several studies of rates of larval development at different temperatures, (Brust, 1967; Hanec and Brust, 1967; Rosay, 1973; Shelton, 1973).

Culiseta (Culicella) morsitans dyari (Coquillett, 1902)

There are records of *Cs. morsitans sens. auct.* from all the provinces and territories of Canada, and across the northern United States, but some records may have been *Cs. s. minnesotae*. Unlike the

Palearctic subspecies of *Cs. morsitans*, which overwinters in the larval stage, (Wesenberg-Lund, 1921), the Nearctic subspecies, *Cs. m. dyari*, overwinters in the egg, (Wallis and Whitman, 1968; Morris, Zimmerman and Magnarelli, 1976). Mattingly (1972) suggests that both the palearctic and the nearctic subspecies overwinter as eggs or as larvae depending on conditions.

Larvae have been taken in *Carex* marshes, lake margins, coastal marshes and bogs, (Jenkins, 1948), and also in woodland pools, (Price, 1961). In New York larvae were found from March to June, the first adults in June, the first pars in July, and only pars after mid-August. Larvae were hatched from dry leaves and soil taken in January, (Morris et al, 1976). In Minnesota larvae were taken from mid-April to late May, (Price, 1961). The larvae of the European form move to the bottom of their ponds in winter and are killed if all the water freezes, (Wesenberg-Lund, 1921).

Blood-meal identifications from *Cs. morsitans sensu lato* in England, (Service, 1971), and New York, (Morris et al, 1976), indicated that nearly all had fed on birds. The European form is said to bite farm animals and man, (Gutsevich et al, 1974). In a field study in Massachusetts almost equal numbers of *Cs. m. dyari* entered mammal-baited and bird-baited traps, but more were engorged in the bird-baited traps, 89 % vs 64 %, (Hayes, 1961). In another field study in Massachusetts, more females were taken in chicken-baited than in rodent-baited traps, and more at 7.6 m than at 1.5 m above the ground, (Main et al, 1966). Eastern encephalitis (EE) virus has been isolated from *Cs. m. dyari* in New York and it may be an important vector of the virus to birds, (Morris et al, 1973).

Culiseta (*Culicella*) *silvestris minnesotae* Barr, 1957

This Nearctic form was described by Barr as a full species and redesignated as a subspecies of the Palearctic *Cs. silvestris* Shingarev by Maslov (Stone, 1967). *Cs. s. minnesotae* is now known right across the northern United States except Alaska (Christiansen, Pinger and Rowley, 1972), and in Alberta (Graham, 1969), Saskatchewan (McLintock et al, 1966), Manitoba (Trimble, 1972), and Ontario (Belton and Galloway, 1966). It has not been recorded from as far north as *Cs. m. dyari*.

In Minnesota adults were taken from April to October, and larvae from mid-May to mid-September, with possibly two generations. Overwintering is likely to be in the adult stage (Barr, 1957; Price, 1961). In Massachusetts almost equal numbers of *Cs. s. minnesotae* entered mammal-baited and bird-baited traps, but the engorgement rate was higher on the birds (81 % vs 64 %), (Hayes, 1961).

2. MATERIALS AND METHODS

2.1. Study areas

Most of the field work was done at two sites, Edmonton (53° 32'N, 113° 30'W) and George Lake (53° 57'N, 114° 06'W), 63 km northwest. Both sites lie in the Mixed Wood Section of the Boreal Forest Zone (Taiga), (La Roi, 1968).

George Lake is shallow and eutrophic. The Department of Entomology field site and the Donalds' Farm are situated at the west end of the lake, (fig. 2 and plate 2a). Most of the field site is covered by a stand of trembling aspen (*Populus tremuloides* Michx.) with the rich shrub and herb layers characteristic of aspen woodland. Other vegetation types include two mats of labrador tea (*Ledum groenlandicum* Oeder) with paper birch (*Betula papyrifera* Marsh.) round their edges, a bog with black spruce (*Picea mariana* (Mill.)) labrador tea and *Sphagnum* moss, and a wet sedge (*Carex sp.*) meadow by the lake shore. The creek draining the lake is blocked by two beaver dams. The Donalds' farm, begun around 1910, now has about thirty buildings of various sizes and a resident population of seven people. There are about 100 cattle and smaller numbers of sheep, a dog, pigs, a goat, cats, rabbits, chickens, geese, ducks and turkeys. Most of the area of the farm is pasture, hay and grain fields.

Wild mammals of seventeen species were seen in the George Lake area during the study and eighteen more would be expected to occur there from the distributions given by Soper (1964). The Rodentia

Fig. 2 (facing page). Map of George Lake study site.

Habitats

A = Aspen woodland

B = Black spruce bog

C = *Carex* meadow

L = *Ledum* mats

P = Lakeside pond

I - VIII = Other pools where *Anopheles*, *Culex* and *Culiseta* larvae were found.

Sampling methods

1 = Catches from cattle

2 = Calf-baited trap

3 = All-night catch from human, 10 - 11/vii/73

4 = Bellamy-Reeves traps, (3 locations)

5 = Searches of flowers (main site)

6 = New Jersey trap, standard

7 = New Jersey trap, modified for live catches

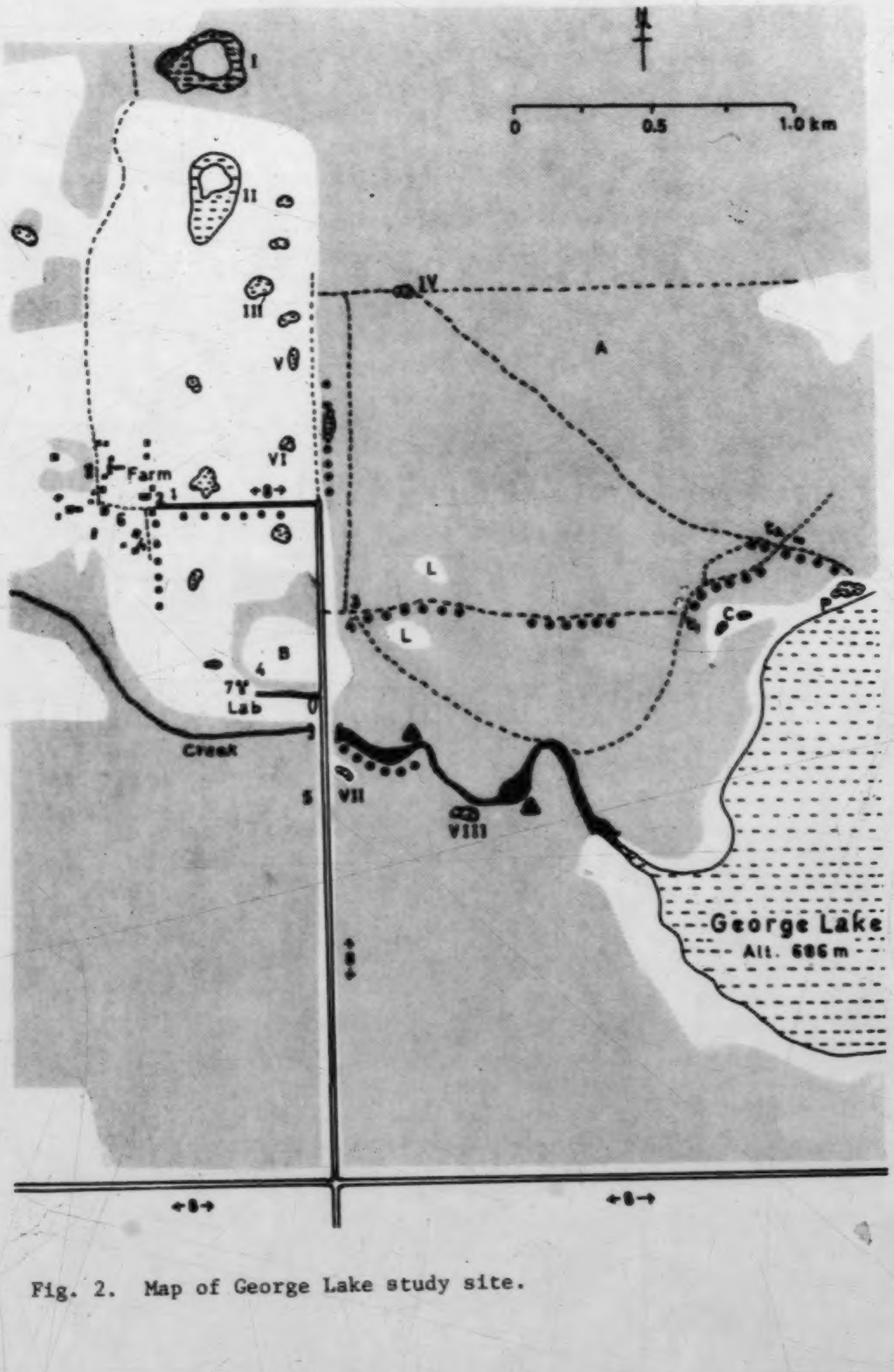
8 = Truck trap route

● = Box shelters (not used in all locations at once; see text)

▲ = Beaver lodges

t_1 = Thermohygrograph, 1973

t_2 = Thermohygrograph, 1974 and 1975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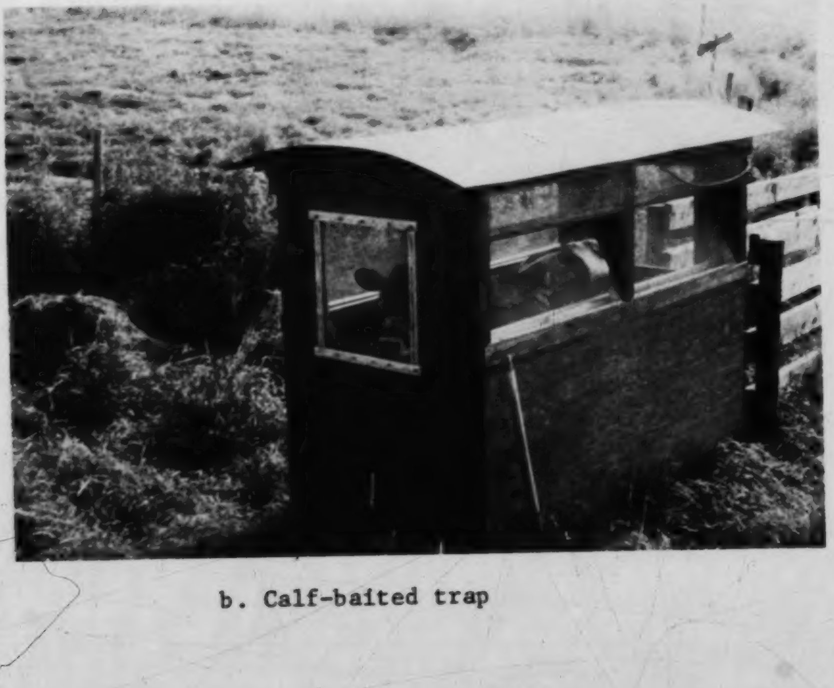
George Lake
Alt. 686 m

Fig. 2. Map of George Lake study site.

Plate 2

a. George Lake field site, view north over the black spruce bog. Trembling aspen in right foreground.



b. Calf-baited trap

are the best represented order, with fifteen species, including Richardson's Ground Squirrel (*Spermophilus richardsonii*). The only Lagomorph, the varying hare (*Lepus americanus*), was scarce during the study period. The three wild Artiodactyla, the white-tailed deer (*Odocoileus virginianus*), Mule deer (*O. hemionus*) and moose (*Alces alces*), were less abundant than the domestic cattle. Other orders represented are the Insectivora (four species), Chiroptera (4), and Carnivora (8). Birds of 65 species have been recorded at George Lake but Dr. Kay Hall (personal communication) estimates that 165 species should occur there, of which the best-represented orders are the Passeriformes (85 species), the Charadriformes (14), and the Anseriformes (18). Garter snakes (*Thamnophis spp.*) are the only reptiles known in the area, and there are 4 species of amphibians, two frogs, one toad and one salamander.

The City of Edmonton in 1975 had an area of 318 km^2 and a population of 452,000 people. The city lies on a plain 665 m above sea level. The North Saskatchewan River and several small tributaries run through the city approximately 50 m below the plain. The river valley system is steep-sided, much of its original woodland has been conserved, and Klassen and Hocking (1964) have argued convincingly that it facilitates the entry of adult mosquitoes to the city. Mosquito control has been attempted for the past 25 years, and the city's present program consists of treating all known or suspected breeding sites to at least two miles (3.2 km) beyond the city limits with larvicides, mainly Dursban, and occasionally applying ultralow volume malathion by helicopter in the river valley system to control adults. Collecting for my own project at Edmonton was confined to two sites on the University of Alberta campus, on the South bank of the river close to the centre of the city.

2.2 Climate and seasons

The climate of the Edmonton region is of the cool temperate type, with cool summers and long cold winters (Longley, 1968). The means of temperature and precipitation recorded at Sion, 3.6 km South of George Lake, during 1941 - 70, (Environment Canada, 1973), are shown in fig. 3. The monthly mean temperatures go from +16.5 C in July to -15.5 C in January, and frosts have been recorded in every month of the year. In this thesis I have divided the year into seasons as follows:

Spring: April and May, (mean temperatures +3.5 to +10.5 C)

Summer: June to August, (mean temperatures +13.5 to +16.5 C)

Fall: September and October, (mean temperatures +10.5 to +5.5 C)

Winter: November to March, (mean temperatures below 0 C).

This differs from the system mentioned by Longley (1968), which restricts winter to the months of December through February. The mean annual precipitation is 490 mm, of which 338 mm is rain and 152 cm is snow (equivalent to 152 mm of rain). There is usually snow on the ground continuously from November to April.

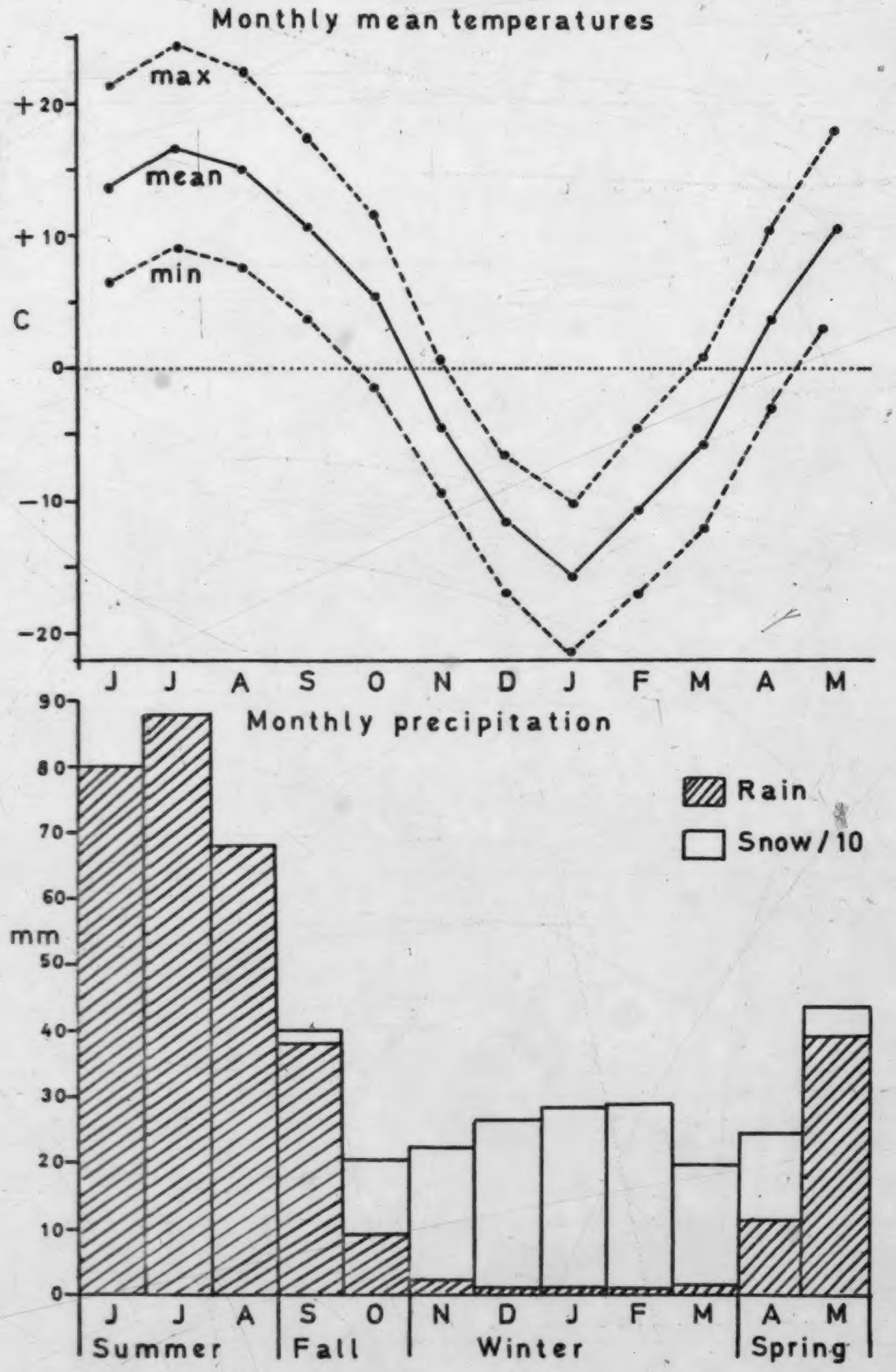


Fig. 3. Normal temperature and precipitation at Sion, Alberta, 1941-70.

2.3 Daylengths, dates and decades

The daylength at 54° N, which is 0°3' North of George Lake, varies from 18:54 on June 21 to 8:50 on December 21 (List, 1958). Here and elsewhere in this thesis the term "daylength" is used to mean the number of hours of daylight, plus twice civil twilight, per 24 hour cycle. Where a value is given the digits before the colon indicate the number of hours and the digits after the colon the number of minutes. For example 14:10 means a daylength of 14 hours and 10 minutes and not a 24 hour cycle with a photophase of 14 hours and a scotophase of 10 hours.

Where dates are given the month is in Roman numerals, between the day and the year. For example, 3 February 1975 would be 3/ii/75. Much of the data will be grouped by thirds of a month, or decades, as follows:

I First decade or early month: days 1 - 10
II Second decade or mid-month: days 11 - 20
III Third decade or late month: day 21 to the last day.

2.4 Collection of adult mosquitoes

For a project of this kind a good collecting method would

a) Collect many *Anopheles*, *Culex* and *Culiseta*, and few *Aedes* or other insects;

b) Detect any mosquito activity from the first snowmelt in spring to freeze-up in the fall;

c) Detect any emergence, blood feeding, and egg development in the population;

d) Collect females alive or fresh enough for dissection; and

e) Give some indication of the relative abundance of each species.

All the methods used were relative in the sense of Southwood (1966) and will have been biassed towards certain species and stages. None can be expected to give an estimate of actual population density per unit area of land. The thirteen collecting methods used may be divided into collections at bait, collections of flying mosquitoes, and collections of resting mosquitoes.

2.4.1. Collections at bait

These collections were intended to sample that part of the population searching for a blood meal, though females were usually caught before they engorged.

Few collections were made from human bait because they rarely yielded *Anopheles* and *Culiseta* females, except in early spring. Mosquitoes were captured, usually by mouth aspirator, as they landed on the collector's bared forearms or lower legs.

Cattle were the usual bait. In 1972 and May 1973 mosquitoes were caught from the windows of the barn in the evenings when the cows were brought there to be milked. Few *Anopheles* and *Culiseta* were taken, and they rarely rested in the barn during the day. From June to September, 1973, and April to early July, 1974, mosquitoes were collected from 10 - 20 unrestrained calves in the open, but when mosquitoes were abundant the cattle would not let the collector go near them, and sometimes stampeded. For the rest of the 1974 and the whole of the 1975 season, collections were made from one calf, haltered and tied to fence posts in the feed lot or pasture. Most catches began at sunset and lasted for one hour, the later part of the catch with the aid of a flashlight. Several bait cattle were used at different times, of various breeds and weighing 150 - 400 kg. The aim was to collect all the mosquitoes that landed on the bait; when there were too many, priority was given to *Anopheles* and *Culiseta*. To compare the results for different seasons, the data were converted to numbers per man-hour of collecting, and they only approximate the numbers per calf per hour for the periods when a single calf was used.

Two stable traps of the "Egyptian" pattern (Bates, 1944), were tried in the hope that they would be useful in measuring biting rates at very low or very high mosquito densities, and in studying diel biting cycles. Each trap (plate 2b) was 2.04 m long, 1.02 m wide and 1.77 m high, with a wooden frame and walls up to 1.0 m high, and the upper walls covered with 1.6 mm mesh flyscreen. On each long side the lower 38 cm of the screened portion sloped inwards by 20 cm to a horizontal entry slit 2 cm wide. A haltered calf was kept in the trap for the first hour after sunset in most of the 1974 catches, and from one hour before sunset to 4 - 5 hours after sunrise in the rest of the 1974 and all the 1975 catches. Mosquitoes were collected by aspirator after the calf was removed.

Three "lard can" traps, (Bellamy and Reeves, 1952) were made from cans 38 cm long and 27 cm in diameter. The ends were replaced by cones of 1.6 mm mesh screen wire going 20 cm inside, with 2 cm holes at their apices. A hole in the side, fitted with a cotton sleeve and a hinged metal door, allowed insertion of the bait and removal of the mosquitoes. The traps were painted inside and out with the same red paint that was used for the box shelters (see 2.4.4). The traps were hung from trees at George Lake, one in the black spruce bog, one in the farmyard and one in the aspen wood near its western edge. In 1974 the bait used was a 1 kg block of dry ice (solid CO_2) wrapped in brown paper and placed in the traps about one hour before sunset. There was usually some left when the traps were emptied 12 hours later, 3 - 4 hours after sunrise. In 1975, adult male and female Japanese Quail (*Coturnix japponicum*) were used as bait, two per trap in a 1 x 2 cm mesh galvanised wire cage, put in the traps 3 - 4 hours before sunset and removed 3 - 4 hours after sunrise. Initially the quail were given neither food nor water in the traps, but three died. No more died after both food and water were provided. Mosquitoes were removed from the traps by aspirator, or by enclosing the entire trap in a plastic bag with a wad of cotton soaked in ethyl acetate, to knock the mosquitoes down.

2.4.2. Collections of flying mosquitoes

Two New Jersey light traps (Haussher's Machine Works, Toms River, N.J.), were used for three consecutive summers. One trap was run at George Lake near the corner of a feed lot, in an area often overgrown with tall weeds. Cattle came to drink at a trough about 3 m away. The other trap was run at Edmonton in the Moss Forest Preserve, a fenced area of almost undisturbed vegetation immediately north of the University of Alberta campus. The trap was hung from the supporting frame of a shed built on the North-facing slope, in a stand of spruce with little undergrowth. Both traps were equipped with 25 watt frosted incandescent lamps, switched on at sunset and off at sunrise by timers. The killing agent in the jar was a 2 cm square of plastic impregnated with dichlorvos ("Vapona No-Pest Strip", Shell Chemicals), enclosed in a gauze bag to stop insects sticking to the tacky surface. One square was effective for about 60 nights in keeping the mosquitoes sufficiently immobilised to remove and sort them. Complete kill was undesirable as the dead mosquitoes rapidly became unfit for dissection. The two standard traps were used for not less than 6 nights per decade at George Lake from 31/iii/73 to 31/x/73, 3/v/74 to 9/x/74, and 14/v/75 to 1/x/75, and at Edmonton from 1/iv/73 to 31/x/73, 10/v/74 to 31/x/74 and 9/v/75 to 31/x/75.

Another New Jersey trap was modified to keep the captured mosquitoes alive, following the design of McLintock (1947), by transferring the fan from the top to the bottom of the cylinder, and putting a removable wire mesh cage above it, connected by a cotton sleeve to a collecting funnel at the top. This modified trap was used for 4 nights in 1974 and 8 in 1975, in an open grassy area north of the laboratory at George Lake.

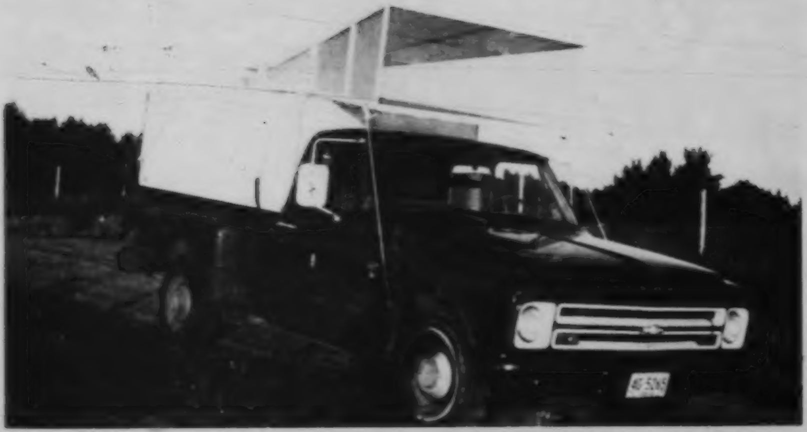
A Malaise trap, as modified by Townes (1962) was operated in the Moss Forest Preserve, in an aspen-dogwood stand, from April to September, 1972.

A truck trap (Bidlingmayer, 1966, 1974) was tried at George Lake in 1975 to see if it would be a useful method of collecting live *Anopheles*, *Culex* and *Culiseta* females for dissection and experimentation, and for studying their flight periodicities. The trap (plate 3a) was made from 1 mm mesh fibreglass screen on a hardwood frame, with a front opening 1.67 m wide by 0.6 m high (1.0 m^2), tapering back 3 m to a 10 cm square opening at the tail, over which was placed a wooden cage with a plexiglas window on one side. A fibreglass screen cone with a 2 cm hole at the entrance to the cage stopped the captured mosquitoes from escaping. Mosquitoes were withdrawn by aspirator through a stockinette sleeve at the rear of the cage, and the side of the cage opposite the window could be quickly opened to get rid of unwanted insects. The trap was mounted on the roof of a truck, with the middle of the entrance 2.12 m above the ground. The truck was driven at 20 - 25 mph (32 - 40 km/h) over a 3.2 km route going down as far as the crossroads at the southeast corner of the University's land, (see map, fig. 2), later extended to about 5 km by going 0.8 km east and 0.8 km west from the crossroads.

2.4.3. Collections from flowers

Mosquitoes taken on flowers were presumed to be feeding on or searching for nectar. Collections from flowers are included in this section because I intend to restrict the term "bait catches" to those mosquitoes taken at animals and dry ice. Most of the collections from flowers were made at night with a flashlight and aspirator, usually in the second hour after sunset, because the first hour was needed for

Plate 3



a. The truck trap at George Lake

b. The lakeside pond, George Lake, July 1975.

collecting from cattle. The most searched area was along the sides of the road going north into the George Lake site as it was there that the most productive plants (*Solidago* and *Tanacetum*) were commonest. A few mosquitoes were collected from flowers in the aspen woodland during the daylight hours. Records were kept of flowering dates of the plant species known or likely to be visited by mosquitoes.

2.4.4. Resting Collections

All the collections of resting mosquitoes were made by aspirator unless otherwise stated. Where the same resting sites were searched repeatedly, the appearance of new mosquitoes provided evidence of flight activity.

A series of 33 windows and 3 doors on Athabasca and Assiniboia Halls, University of Alberta Campus, was the most productive resting site. The windows were 1.9 m high and 1.25 m wide, with sills at ground level and fly screens set 20 cm in from the walls. Twenty-one faced west, 9 north and 5 east. Collections were made usually between 08:00 and 09:00 MDT (1 - 5 hours after sunrise), during September and October in 1972 and 1973, and from April to October in 1974 and 1975.

Twenty-four cubical box shelters, 30 cm on the side, with one side open, were made from plywood and painted inside and out with one coat of red paint, ("Carnival Red", #1-345, Canadian Pittsburgh Industries Ltd.). The value of red paint in increasing the catch of mosquitoes in artificial shelters was first demonstrated by Goodwin (1942) with *Anopheles quadrimaculatus* Say. Samples of the paint exposed to light sources of 300 - 760 nm showed more than 13% reflectance only at wavelengths greater than 600 nm, in that part of the spectrum to which most insects are insensitive, (Wigglesworth, 1972). The box shelters were set on the ground at George Lake with the open sides facing north, in lines of six boxes approximately 30 m apart. Goodwin (1942) got more *Anopheles quadrimaculatus* in north-facing boxes than in boxes facing south, east or west. In 1974 one line was placed in the farmyard and later shifted to the side of the driveway where there was no other shade or shelter; the second line was along the western edge of the aspen stand, later shifted to the lake shore; the third line was inside the aspen stand, and the fourth line was on the creek bank. In 1975 all the box shelters were in the aspen stand, beside the main track running down to the lake shore. The box shelters were examined on 60 days in 1974 and 25 days in 1975, usually between 09:00 and 13:00 hours. Plants were cut short round the open sides of the boxes and the many spiders found in the boxes were killed.

A few mosquitoes were trapped emerging from badger burrows during summer (see Chapter 7). A variety of other resting sites searched at irregular intervals yielded mosquitoes. A culvert under the road was a good site but could not be searched when the water was high or fast. Of the many buildings in the farmyard the most consistently productive were two sheds near the pasture, one for sheep and the other for pigs. Rockpiles and logpiles where the females overwintered yielded a few of the same species in summer, though they were not regularly searched. In the aspen stand by the lake a few *Culiseta inornata* were found resting in the Stevenson Screen, and a few *Anopheles earlei* were found in a trailer and under a boat. Sweep netting in the aspen stand and at the edges of fields, and searches around tree bases, yielded only *Aedes*, or no mosquitoes at all.

2.5. Identification and voucher specimens

The main reference for identifications was Carpenter and LaCasse (1955). Barr (1957) and Price (1958) were used to identify *Culiseta silvestris minnesotae* Barr. For nomenclature I have followed Stone, Knight and Starcke (1959) and supplement III (Stone, 1967). Voucher specimens of males, females and larvae of all the *Anopheles*, *Culex* and *Culiseta* species (except *Culiseta impatiens* of which only two males were taken), and some of the *Aedes* species, are being deposited in the Strickland Museum, Department of Entomology, University of Alberta, and in the Canadian National Collection, Ottawa.

2.6. Storage, dissection and measurement of adults

Live adults were transported in plastic cups covered with gauze or in cages 15 x 2.5 x 2.5 cm, with the sides and ends of plexiglas and the tops and bottoms of mosquito netting (Trpis, 1968). The cups and cages were wrapped in moist paper towels, put in polyethylene bags and stored at +2 C. The mosquitoes were dissected as soon as possible after collection, but some collected in August and September had to wait a month. Survival was good if the mosquitoes' wings did not get trapped in condensation on the walls of the cage. Some fatbody depletion and ovary development may have occurred during storage at +2 C, but larval muscle remnants and nectar-filled crops were found after a month under these conditions. Mosquitoes stored in the freezer at - 18 C in sealed vials, with a small strip of paper towelling soaked in water, could be dissected to determine fatbody development and parity, but details of the follicles were obscured. Mosquitoes which dried before freezing or while frozen were almost useless for dissection.

The abdomens of females were snipped off, wetted by a quick dip in 70% ethanol, and dissected in saline (0.7% NaCl in deionised water) using watchmakers' forceps and a surgeons' needle. When specimens were dissected in plain deionised water the ovarian follicles swelled rapidly, making the cellular details easier to see but introducing the danger of overestimating the lengths of the follicles. The follicles also swelled in the saline, but much more slowly; all ovary and follicle measurements are from dissections in saline. The dissections were done at one end of a microscope slide under a Leitz stereo dissecting microscope at X25, with transmitted light. At this magnification the abdomen was examined for meconium, muscle remnants, crop contents, and fatbody development. The length of one ovary was measured at X25 using an eyepiece micrometer (1 division = 50 micrometres). Then the distal end of one ovary was torn open, a few follicles were teased out, the magnification was increased to X150 and the lengths of a typical follicle and its germarium were measured, (1 division = 8.3 micrometres). The follicles were assigned to a stage of Christophers, (see section 2.7.1.). The ovaries were washed off in deionised water and left to dry on another slide. The spermathecae were transferred to a drop of saline at the other end of the slide and crushed with a cover glass. Examination for sperm was made under a Leitz compound microscope at X200. The dissection examination, measurement and recording took an average of 5 minutes per mosquito. The surface tracheation of the dried ovaries was examined under the compound microscope at X200 to determine if the mosquito was parous, (Detinova, 1962).

2.7. Terminology used in describing dissections

2.7.1 Stages of development of the ovarian follicles

Christophers (1911) divided the development of the ovarian follicles in *Anopheles* into five stages. Mer (1936) introduced stage N to cover earlier phases of follicle differentiation and stage I-II to cover the deposition of the first yolk granules. Several other modifications have been published. I used the following version, based mainly on Detinova (1962, fig. 1 and page 17), with the subdivisions of stage N based on Oda (1968) and Kawai (1969). The stages are also shown in plate 4 and Fig. 4.

N1: The follicle is not differentiated from the germarium which is cigar shaped.

N2: A constriction is visible as the follicle buds off from the germarium.

I: The follicle is nearly spherical with a distinct epithelium on the outside and eight cells within.

I-II: One of the eight inner cells is recognizable as the oocyte by its larger nucleus, with a crown of yolk granules surrounding it.

Some stages of follicle development in *Culiseta inornata* (legend for Fig. 4 and Plate 4).

All live preparations in 0.7% NaCl, from nulliparous, laboratory-reared females. Phase contrast, all at X200 except for stage III (bottom right), X100.

N1.	1 hr old (after eclosion)
N2.	2 hr old
I.	2-3 days old
I-II.	6-7 days old
IIa.	8-9 days old
IIb.	6-7 days old, 2 days after a small blood meal
III.	(bottom L). 15-20 days old, 30 hr after a blood meal
III.	(bottom R). 15 days old, 4 days after a blood meal, almost in stage IV. Secondary follicle in stage N2.

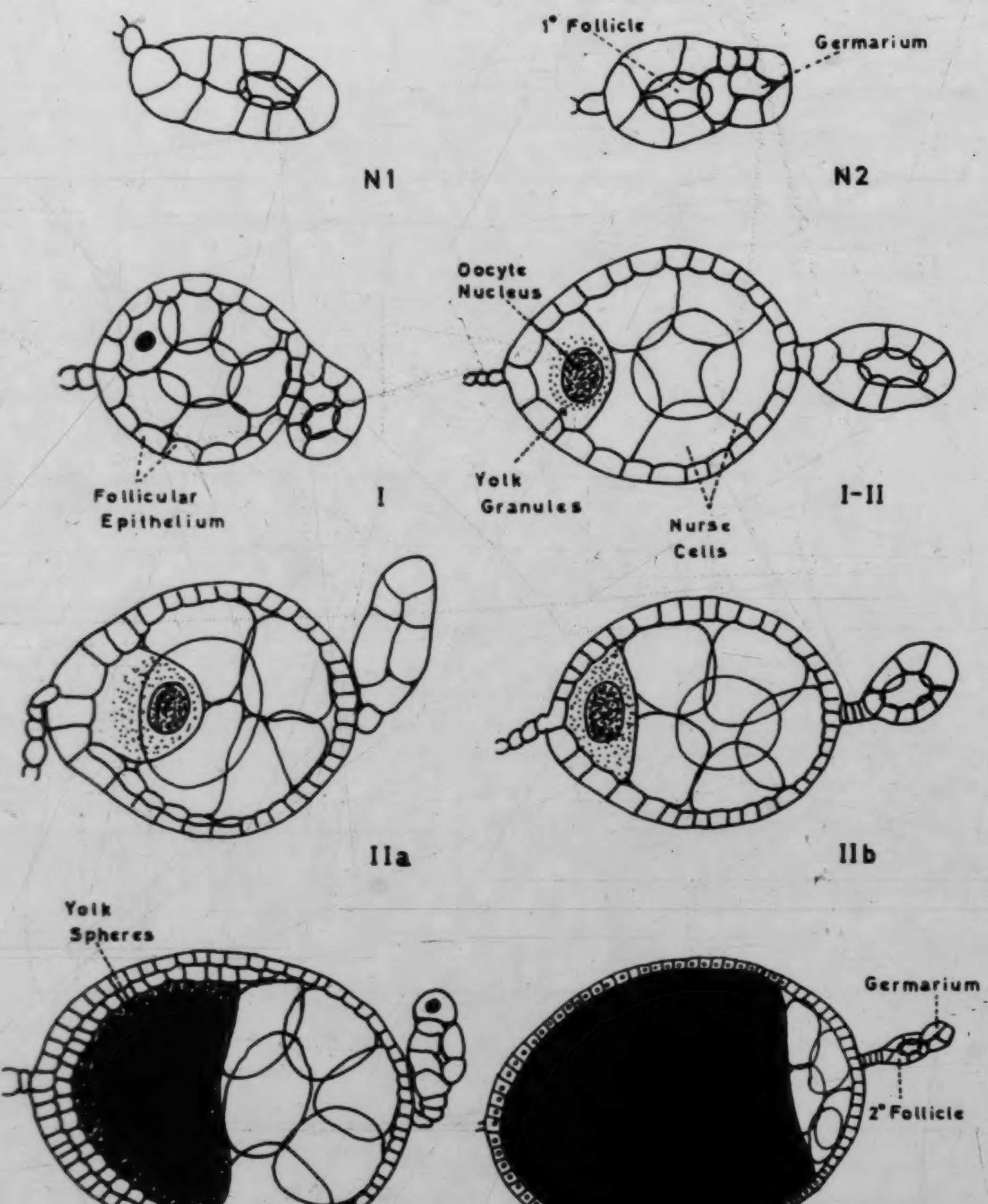
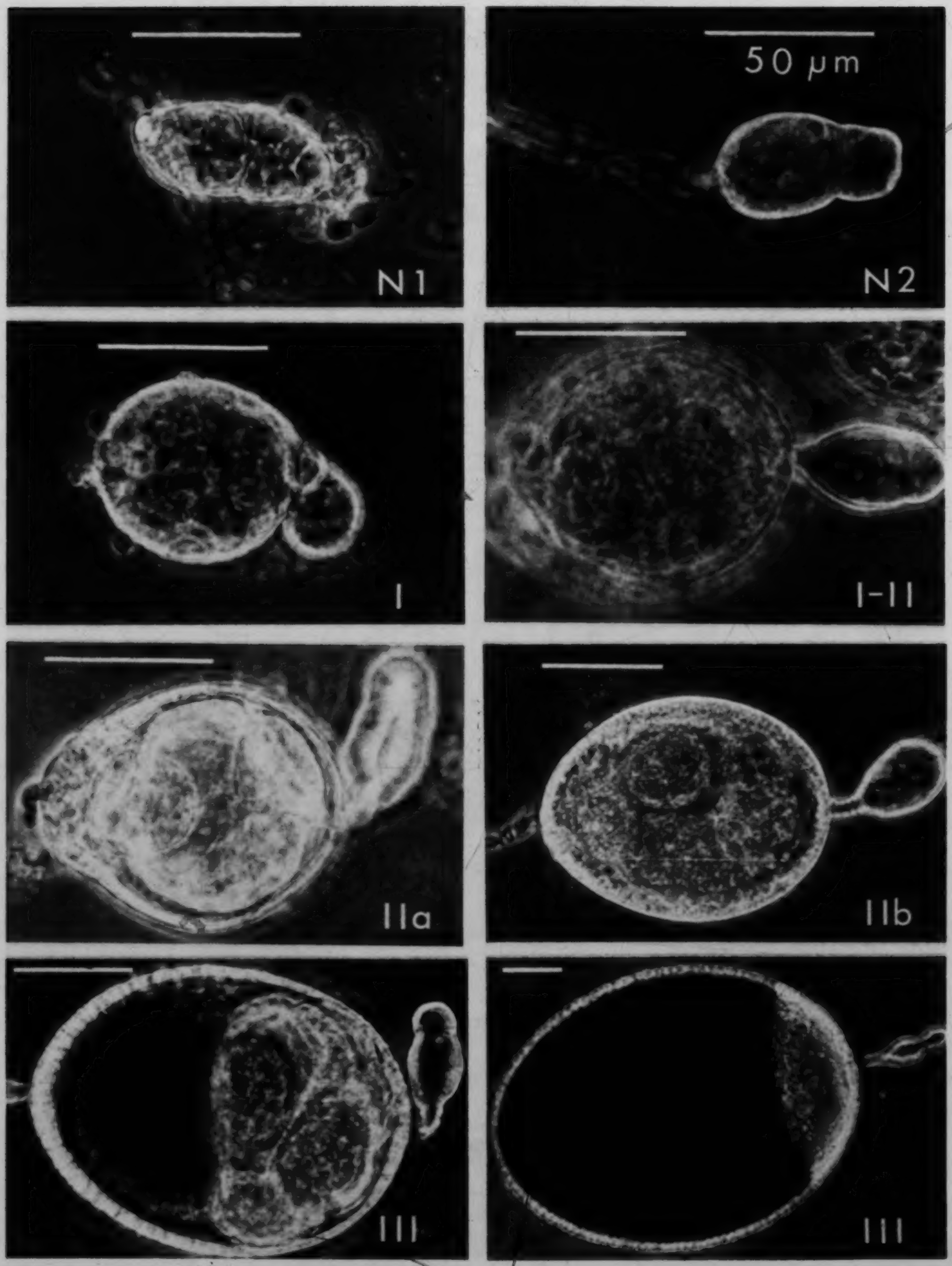

1° Follicle
Germarium
N1
N2
Oocyte Nucleus
Follicular Epithelium
I
Yolk Granules
Nurse Cells
I-II
IIa
IIb
Yolk Spheres
Germarium
2° Follicle
III
III

Fig. 4. Some stages of follicle development in *Culiseta inornata*, (interpretative drawings of material shown in plate 4).

Plate 4


50 μm
N1
N2
I
I-II
IIa
IIb
III
III

Some stages of follicle development in *Culiseta inornata*.

IIa: The oocyte has yolk granules scattered throughout its cytoplasm, is rounded where it abuts the nurse cells, and occupies about one fifth of the follicle.

IIb: The oocyte has more densely-packed yolk granules, is flat where it abuts the nurse cells and occupies up to one half of the follicle.

III: The oocyte occupies one half to three-quarters of the follicle, which is elongated.

IV: The oogonium is full of yolk, and occupies about nine-tenths of the greatly elongated follicle.

V: The follicle contains a mature egg, ready to be laid.

Mer (1936) defined the stage N follicle as "consisting of undifferentiated cells", and Detinova (1962) interpreted this as meaning the stage when the oocyte is not yet distinct from the seven nurse cells, but with the follicular epithelium distinct around them. The paper that both Detinova (1962) and Bertram (1962) cite for the origin of the term "stage N" is Mer (1932) but I cannot find the term anywhere in it. Oda (1968) and Kawai (1969) used "No-1" and "No-2" for the stages of budding of the follicle from its germarium, and divided differentiation of the oocyte and the beginning of yolk deposition into additional stages N, Ia, Ib, and I-II. I could not distinguish the oocyte from the nurse cells until the stage (I-II) when a crown of yolk granules had appeared round its nucleus. This stage could sometimes be seen in *Anopheles earlei* and *Culiseta alaskaensis* under the dissecting microscope at X150; such individuals are included with those in stage I for analysis. Stage I-II was seen in *Culiseta* *inornata* only with phase contrast at X400. My stages N1 and N2 correspond to stages No-1 and No-2 of Oda (1968) and Kawai (1969). In the dissections of wild caught females, viewed at X150, my stage I covers stages N, I, and I-II of Detinova (1962) and stage IIa of Clements (1963). My stage IIa corresponds to "early stage II" of Hitchcock (1968) and my stage IIb to his "full stage II". Stage N in this account means stages N1 and N2 combined and stage II means stages IIa and IIb combined.

2.7.2. Parity and nulliparity

A female was considered nulliparous (never to have laid eggs before), or a nullipar, if the tracheoles on the surfaces of her ovaries were tightly coiled in skeins, and to be parous (to have laid eggs at least once before), or a par, if the tracheoles were uncoiled and spread out in a loose network over the surface of the ovary, (Detinova, 1962). Polovodova's method of determining the number of egg batches laid, by counting the number of dilatations on the ovariolar stalks, (Detinova, 1962) was not attempted in this study. The midguts of *Anopheles maculipennis* that have been stretched by a blood meal can be distinguished by an uncoiling of the tracheoles similar to that which occurs in the ovaries, (Detinova, 1962). I tried to use this technique with *Culiseta inornata* but could not identify a series of unknowns prepared by another student.

2.7.3. Fatbody ratings

The development of fatbody in the abdomen was rated according to the scheme of Burdick and Kardos (1963), as follows:

> "0: no obvious fat deposits and a flat abdomen;
>
> 1: definite fat development around spermathecae and occasionally small amounts near the ovaries (abdomen still flat or with the posterior segments slightly enlarged);
>
> 2: fat bodies around the ovaries and the rest of the abdomen filled but not distended; and
>
> 3: abdomen distended by fat."

2.7.5. Crop contents

The crop (ventral diverticulum) in mosquitoes is used to store sugar meals, (Clements, 1963). The crops were rated as follows:

E: empty, (collapsed),
G: gas bubbles only,
S: syrup only,
S+G: syrup mixed with gas bubbles.

2.7.6. Stages of the gonotrophic cycle

Sella (1920) divided the gonotrophic cycle in *Anopheles* into 7 stages based on the external appearance of the abdomen. I have not seen Sella's paper and have followed the version of the scheme given by Detinova (1962). Unfed mosquitoes, with no external sign of blood in the gut are in stage 1, mosquitoes with a fresh blood meal but no visible egg development are in stage 2, mosquitoes with partly digested blood meals and partly developed eggs are in stages 3 to 6, and mosquitoes with mature eggs and no trace of blood in the gut are in stage 7. When the crop was full the midgut and developing ovaries were forced back, making it difficult to follow Sella's scheme exactly. Since few females in stages 2 to 6 were collected in this study, the results are mostly presented as unfeds (stage 1), feds (stages 2 to 6) and gravids (stage 7).

2.7.6. Teneral and diapausing females

Mosquitoes with muscle remnants in the abdomen visible at X25 were classed as teneral, or newly-emerged, (Rosay, 1961). Other features of the newly-emerged mosquito, such as presence of meconium (greenish-grey material) in the midgut, and lack of insemination, were noted, but the division into tenerals and post-tenerals was based only on the presence or absence of muscle remnants.

A female was considered in reproductive diapause if she was post-teneral, her follicles had not reached stage IIa, and the F:G (Follicle:Germarium) ratio did not exceed 1.5 or 2.0, depending on species.

2.8. Analysis of data

The results of dissections of 925 *Anopheles earlei*, 844 *Culex territans*, 1030 *Culiseta alaskaensis*, 3575 *Cs. inornata* and 280 *Cs. m. dyari* were analysed with the aid of a computer. The data in the notebooks were coded and transcribed to Fortran coding forms, punched cards and magnetic tape. The combined error rate for the two human stages of transcription, (coding forms and card punching) was estimated by double checking a randomly selected printout of 100 dissection records from the tape against the original notes. The error rate was 18 in 100 cases (18%) or 18 in 1669 characters (1.1%). Thus, in a cross-tabulation with 5 levels of selection, the probability of any case being placed in the correct box was $(1 - 0.11)^5$, or 0.936. Such a high error rate would not have been tolerated if I had not been familiar with the original data. The results for 254 cases which contributed to some odd-looking results were printed out in full and the tables corrected by hand where necessary.

The data on the tape were sorted into files by species, then analysed on the University of Alberta's IBM 360 and Amdahl 470 computers, using the "crosstabs" and "breakdown" subprograms from the Statistical Package for the Social Sciences (Nie *et al*, 1975). Since the emphasis here is on seasonal changes, most of the output consisted of cross-tabulations by months and decades. Before the tables were printed the records were usually sorted by first removing all tenerals, feds and gravids, then dividing into nullipars at bait, nullipars at other sites, pars at bait and pars at other sites.

Williams' Mean (Haddow, 1960) was used wherever the numbers in individual catches in a series covered a wide range.

Wherever possible times of day are stated in relation to time of sunset rather than as clock times, because of the great changes in daylength during the season. Time intervals close to sunset are expressed in multiples of civil twilight or "Crep" units, (Nielsen, 1961).

2.9. Blood meal identifications

The blood meals of females in Sella stages 2 to 5 were quick-dried by smearing the midguts on filter papers, or by putting intact mosquitoes in a dessicator with Drierite (anhydrous calcium sulphate, from W. A. Hammond, Xenia, Ohio), at 22 C. The dried samples were sent to Dr. J. D. Edman and Mrs. H. Lynn, Florida Medical Entomology Laboratory, Vero Beach, Florida for identification. The filter papers were soaked in saline to redissolve the midgut contents. In all other respects the blood meal identifications followed the precipitin test described by Edman (1971). Samples reacting with non-specific mammal antisera were retested against bovine, pig, rabbit, rodent, human, dog, and cat, and those reacting with non-specific bird antisera were retested against passerine, ciconiiform, chicken and hawk. Spots of blood dried on filter papers from porcupine and Richardson's ground squirrel in the Edmonton area, did not cross-react with any of the specific antisera used.

2.10. Virus testing

Mosquitoes collected during the summer of 1975, mainly at George Lake, and blood samples from the quail used as bait in the Bellamy-Reeves traps were tested for arboviruses by Dr. O. Morgante, Provincial Laboratory of Public Health, Edmonton. The mosquitoes were killed by freezing for about one hour, quickly sorted into species and divided into pools of 1 to 60 individuals, which were placed in sterilized screw cap vials sealed with several layers of surgical adhesive tape. The vials were wrapped in two polyethylene bags sealed separately with tape and delivered on dry ice (-78 C). The quail, which came from a laboratory colony at the University of Alberta, were bled from one wing before and after the 4 month period they spent at George Lake, where they were never protected from mosquito attack. The blood samples were delivered fresh and unfrozen. The virus tests followed Shemanchuk and Morgante (1968) for the mosquitoes and Morgante, Shemanchuk and Windsor (1969) for the quail blood. Dr. Morgante mentioned (pers. comm.) that haemolysis of the post-exposure quail blood samples may have interfered with the tests.

2.11. Anthrone tests for nectar

In 1973 and 1974 some mosquitoes were tested for nectar using anthrone reagent, as described by Van Handel (1972).

2.12. Habitat, collection, preservation and identification of larvae

The larval habitat most searched was a permanent pond by the shore of George Lake, the "Lakeside Pond", (Fig. 2, Plate 3b). From June to September it was shaded by willow trees on the banks, emergent sedges in the shallower parts and a thick covering throughout of duckweeds (*Lemna minor* L. and *L. trisulca* L.). In 1972 and 1973 the pond had an area of 1200 m^2 and a maximum depth of approximately 1.0 m, and it was separated from the lake. Heavy snowfall in the winter of 1973-74 and rains in July 1974 raised the water level by 35 - 60 cm, the pond overflowed its banks, and the water reached to the edge of the woods during the rest of 1974 and 1975. At least once per decade from May to October during 1973, 1974 and 1975, the pond was searched for larvae using an 800 ml dipper, from the banks in 1973, and from a boat or by wading after the banks overflowed. No attempt was made to search the whole area evenly, and most attention was devoted to those parts that had already produced larvae, because the main aim was to determine the presence or absence of species and instars, and the subsidiary aim was to obtain a rough idea of relative abundance. Occasional searches of other pools in the George Lake area were made. *Anopheles*, *Culex* or *Culiseta* larvae were found only in permanent or semipermanent pools with emergent vegetation.

In 1973 the live larvae were assigned to instars by comparison with laboratory-reared larvae of known instar, then reared through to the adult stage to determine the species. In 1974 and 1975 all larvae were counted at the time of collection, then killed and preserved and their instars determined by comparing the widths of the head capsules with those of laboratory-reared larvae of known instar. Pupae were identified by letting the adults emerge. Larvae were killed by dropping in hot water and preserved in MacGregor's Solution (Lane, 1974), a mixture of formalin, borax and glycerol.

2.13. Recording of air and water temperatures at George Lake

During 1973-75 air temperatures were recorded continuously from May to October with a thermohygrograph (Fuess, Berlin), in a Stevenson Screen, situated in the *Carex* meadow by the lake in 1973 (t_1 in Fig. 2) and about 150 m into the aspen stand in 1974 and 1975 (t_2). Water temperatures in the lakeside pond were measured continuously over the same period using battery-driven temperature recorders with bourdon tube probes (Palmer Instruments, Cincinnati, Ohio). In 1973 the probe was fixed at a depth of 10 cm in an area where the water depth remained at about 20 cm throughout the season. In 1974 and 1975, when the water level varied from 55 to 100 cm at the same spot, the probe was suspended 2.5 cm below the surface and protected from insolation by a styrofoam float. During August 1974 and from June to September 1975, a second recorder was used with the probe at the bottom of the pool, in the same spot.

2.14. Laboratory culture and maintenance of *Culiseta inornata*

Two colonies were maintained, following the methods of McLintock (1952, 1964). The parent stock for each colony was a group of 30 - 50 females collected on the University of Alberta campus. The Edmonton I colony was founded in September 1971 and destroyed in September 1974, and the Edmonton II colony founded in August 1974. There were about 8 generations per year.

The rearing rooms did not normally receive natural daylight and were lit for 16 hours per day by a 40 W incandescent or a 15 W cool white fluorescent lamp, with extra lighting during working periods. The temperature was 20 ± 2 C, and the humidity uncontrolled and low. Newly-hatched larvae were placed in lots of up to 1000 in round, white enamel bowls 19 cm in diameter and 7.6 cm deep, with 1.25 l of Bates' Medium S, then transferred 5 days after hatching in lots of 200 to white plastic trays 35 x 23 x 6.3 cm deep, with 2 litres of Bates' Medium. Air was bubbled continuously through the media. Larvae were fed either a mixture of laboratory rabbit chow and baker's yeast

(2:1 v/v), or "Tetramin" tropical fish food (Baensch, West Germany). The food was ground and passed through a 0.425 mm sieve, and sprinkled on the water surface. Pupae were harvested with a pipette and placed in small bowls of water with cork rafts in the adult cage.

The adults were kept in wooden glass-fronted cages 45 x 30 x 28 cm high. Humidity was raised by covering the flyscreen roofs of the cages with paper towels wetted with water siphoned from inverted jars. Gauze bags of soaked raisins were hung in each cage. A few females were removed weekly to a 250 ml beaker covered with gauze, and allowed to take blood from my arm, then returned to the cage. A water-filled pan painted red inside was provided for egg laying.

Small lots of females for experiments were held in plastic cups or tubes lined with paper towelling and covered with gauze. These containers were held either in a metal cupboard in the rearing room, with portholes to admit light and pans of water to raise the humidity, or in incubators accurate to ± 1 C, (Sherer 25-7 or Precision Scientific 815), lit by two 40 W or one 15 W cool white fluorescent lamp. In some experiments the tubes of mosquitoes were covered with wet paper towels and clear polyethylene sheets. In others the tubes were placed in plywood boxes 44 x 29 x 16.5 cm high within the incubators. Each box was lit by two 6 v, 0.15 A incandescent lamps mounted in the lid and powered from a microscope lamp transformer. Humidity in the boxes was raised by two water-filled 125 ml flasks with wicks of paper towelling.

Since all lighting in the laboratory was on a 24-hour cycle, only the daylength (the duration of the photophase) is indicated. For example, 16 hr/20 C denotes 16 continuous hours of light and 8 continuous hours of darkness per day, with no twilight, and a constant temperature of 20 C.

3. FIELD OBSERVATIONS, APRIL TO OCTOBER

3.1. Collections of Adults

3.1.1. General

The numbers of adults collected from April to October inclusive during 1972 - 75 are shown in table 2. Of a total of 125,979 adults, only 17.85% were *Anopheles*, *Culex* or *Culiseta*, and 73.8% were *Aedes vexans*. These totals do not include females collected emerging from burrows in April and May, and resting in root cellars in September and October, or any collected in winter (see Chapter 7). A few other collections made in 1971, 1972 and 1976 are not included in the totals, but have been added to the sections on individual species where useful.

The bait catches never yielded any *Culex* females and the *Aedes* always outnumbered the *Culiseta* and *Anopheles*, even though some of the catches from calves were selected for *Culiseta*, (Table 3). The flowers yielded more than 50% *Anopheles*, *Culex* and *Culiseta* but were biassed towards these genera because most collections were made late in the year. *Aedes vexans* predominated in the New Jersey traps but useful numbers of *Cs. inornata* were also obtained. The truck trap yielded few *Anopheles*, *Culex* or *Culiseta*. The windows were a good source of *Culex territans*, *Culiseta alaskaensis* and *Culiseta inornata*, while the box shelters and other resting collections were a good source of *Anopheles earlei*.

A few of the *An. earlei* were taken at bait b[illegible]e best sources

Table 2. Total numbers of adult mosquitoes collected at George Lake and Edmonton in spring, summer and fall, 1972-75.

		George Lake	Edmonton	Total	%
Anopheles (Anopheles) earlei Vargas	F	867	107	974	0.77
	M	599	27	626	0.50
Culex (Culex) tarsalis Coq.	F	2	22	24	0.02
	M	0	10	10	0.008
Culex (Neoculex) territans Walker	F	219	1,382	1,601	1.27
	M	194	794	988	0.78
Culiseta (Culiseta) alaskaensis (Ludlow)	F	722	705	1,427	1.13
	M	165	447	612	0.48
Cs. (Cs.) impatiens (Walker)	M	1	1	2	0.002
Cs. (Cs.) inornata (Williston)	F	9,176	5,297	14,473	11.49
	M	620	453	1,073	0.85
Cs. (Culicella) morsitans dyari (Coq.)	F	169	309	478	0.38
	M	74	102	176	0.14
Cs. (Culic.) silvestris minnesotae Barr	F	3	2	5	0.004
	M	30	12	42	0.03
Aedes (Aedes) cinereus Meigen	F	71	47	118	0.09
	M	52	21	73	0.06
Aedes (Aedimorphus) vexans (Meig.)	F	69,382	15,887	85,269	67.67
	M	5,473	2,257	7,730	6.13
Ae. (Ochlerotatus) campestris (D. and K.)	F	15	1	16	0.01
Ae. (O.) canadensis (Theo.)	F	100	21	121	0.10
Ae. (O.) dorsalis (Meig.)	F	901	10	911	0.07
Ae. (O.) excrucians (Walker)	F	169	49	218	0.17
Ae. (O.) fitchii (F. and Y.)	F	1,311	342	1,653	1.31
Ae. (O.) flavescens (Muller)	F	379	27	413	0.33
Ae. (O.) riparius (D. and K.)	F	43	4	47	0.04
Ae. (O.) spencerii (Theo.)	F	973	17	990	0.79
"Black-legged" *Aedes*	F	3,348	370	3,718	2.95
Aedes spp. indet.	F	209	14	223	0.18
	M	1,046	804	1,850	1.47
Coquillettidia perturbans (Walker)	F	90	1	91	0.07
Culicine spp. indet.	F	2	0	2	0.002
	M	32	0	32	0.025
TOTAL		96,437	29,542	125,979	99.321

Table 3. Percentages of mosquitoes of different species captured by different methods, 1972-75.

		At Bait						Flying		Resting		
		Human	Cattle (s)	Calf-baited trap	Quail-baited trap	Dry Ice trap	On flowers	N.J. traps	Truck trap (s)	Windows	Box shelters	Others[1] (s)
Total collected		2744	5998	4076	243	262	576	102,791	1260	5919	1279	349
Anopheles earlei	F	0.04	0.53	0.44	0.00	0.38	0.17	0.23	0.71	1.49	33.33	38.89
	M	0.00	0.00	0.00	0.00	0.00	19.97	0.11	0.32	0.24	26.29	9.94
Culex tarsalis	F	0.00	0.00	0.00	0.00	0.00	0.00	0.003	0.00	0.27	0.08	0.29
	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00
Culex territans	F	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.79	20.60	6.88	8.48
	M	0.00	0.00	0.00	0.00	0.00	7.99	0.48	0.32	6.31	1.64	1.46
Culiseta alaskaensis	F	0.07	6.20	2.30	0.82	0.38	0.35	0.30	0.24	10.21	2.03	2.63
	M	0.00	0.00	0.00	0.00	0.00	2.26	0.17	0.24	6.71	1.41	0.29
Culiseta impatiens	M	0.00	0.00	0.00	0.00	0.00	0.00	0.001	0.00	0.00	0.08	0.00
Culiseta inornata	F	0.55	24.17	5.17	0.00	0.38	3.12	9.62	1.11	46.92	5.32	7.02
	M	0.00	0.00	0.00	0.00	0.00	13.02	0.74	0.56	1.79	6.96	8.77
Culiseta m. dyari	F	0.00	0.02	0.02	24.69	0.00	0.17	0.16	0.16	2.57	7.12	0.29
	M	0.00	0.00	0.00	0.00	0.00	0.87	0.09	0.16	0.71	2.97	0.00
Culiseta s. minnesotae	F	0.00	0.00	0.00	0.00	0.00	0.17	0.001	0.00	0.02	0.00	0.00
	M	0.00	0.00	0.00	0.00	0.00	3.82	0.00	0.00	0.00	0.08	0.29
Aedes vexans	F	47.05	48.32	73.74	1.23	38.93	15.97	75.22	30.48	0.30	0.31	0.00
	M	0.00	0.00	0.00	0.00	0.00	15.80	7.18	17.46	0.05	0.00	0.00
other *Aedes* spp.	F	51.82	20.57	17.89	65.85	59.55	10.07	4.06	15.39	1.13	2.37	21.07
	M	0.00	0.00	0.00	0.00	0.00	6.25	1.37	31.98	0.52	0.78	0.58
Coq. perturbans	F	0.47	0.17	0.44	7.00	0.38	0.00	0.03	0.08	0.02	0.00	0.00
Culicini indet.	F	0.00	0.02	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.35	0.00

1 Animal houses, empty buildings, culvert, rockpiles, logpiles, sweeping vegetation.

(s)Some collections selected for *Anopheles*, *Culex* and *Culiseta*.

were resting sites (fig. 5). Although far more *Clx. territans* were collected than *Clx. tarsalis*, the relative numbers collected at different sites were remarkably similar, the best source of both species being the windows. Many of the *Cs. alaskaensis* and *Cs. inornata* were taken from cattle and in the calf-baited trap, and many of the *Cs. m. dyari* in the quail-baited traps. Although the New Jersey traps were a good source of unfed females, particularly of *Cs. inornata* and *Cs. m. dyari*, the proportion of feds and gravids caught was low. The windows and box shelters were much better sources of feds and gravids (Fig. 6). To avoid bias due to the presence of diapausing females late in the season, only the data for the gonoactive season for each species have been included in Fig. 6.

3.1.2. At bait

Table 3 includes data for only one catch from human bait, made by me at George Lake continuously for 11 hours, from 2 hours before sunset on 10/vii/73 to 1 1/4 hours after sunrise the next day. Out of 2744 females there were only 1 *Anopheles earlei*, 2 *Culiseta alaskaensis* and 15 *Culiseta inornata*. Many other captures from human bait were performed at George Lake and Edmonton during 1972 and 1973, for other projects and the proportions of *Anopheles* and *Culiseta* were always low.

The calf-baited trap kept some mosquitoes away from the calf. During 1975 the numbers taken from the calf in the open during the first hour after sunset were usually greater than those taken in the calf-baited trap all night. Half an hour after sunset on 14/v/75,

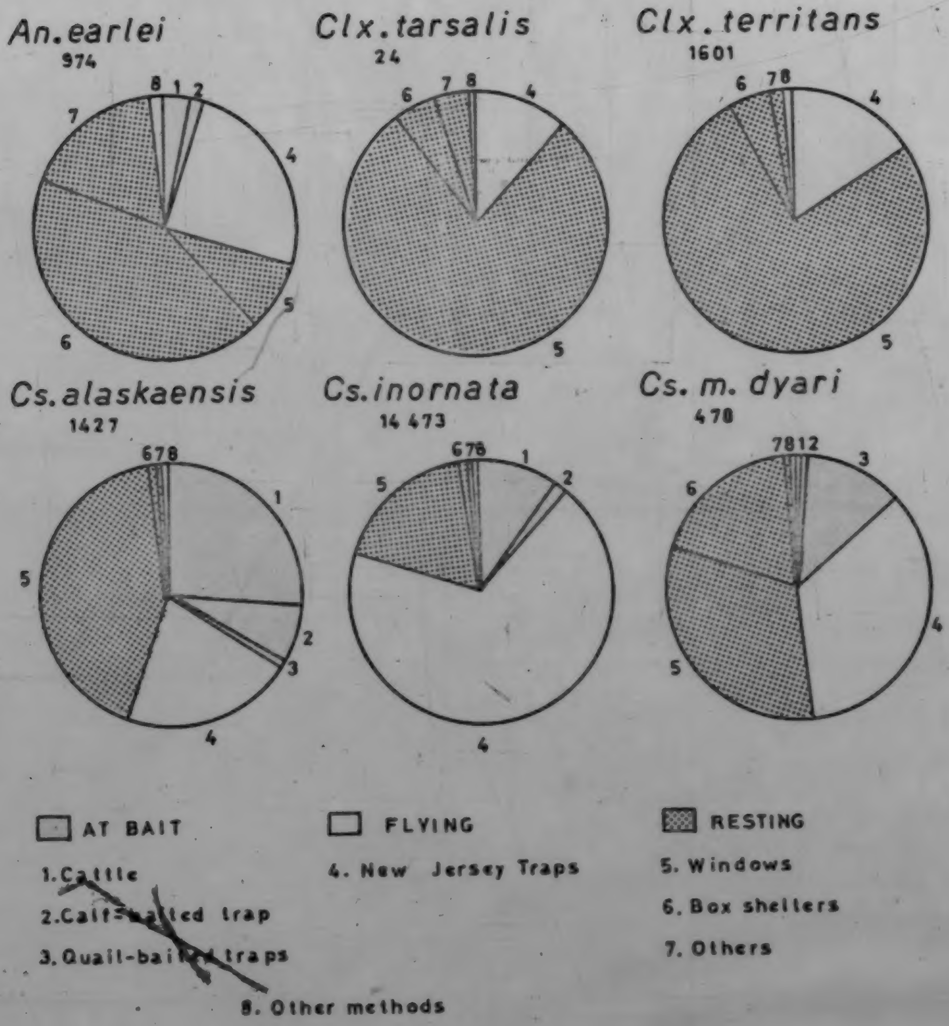


Fig. 5. Chief sources of *Anopheles*, *Culex* and *Culiseta* females, 1973-75.

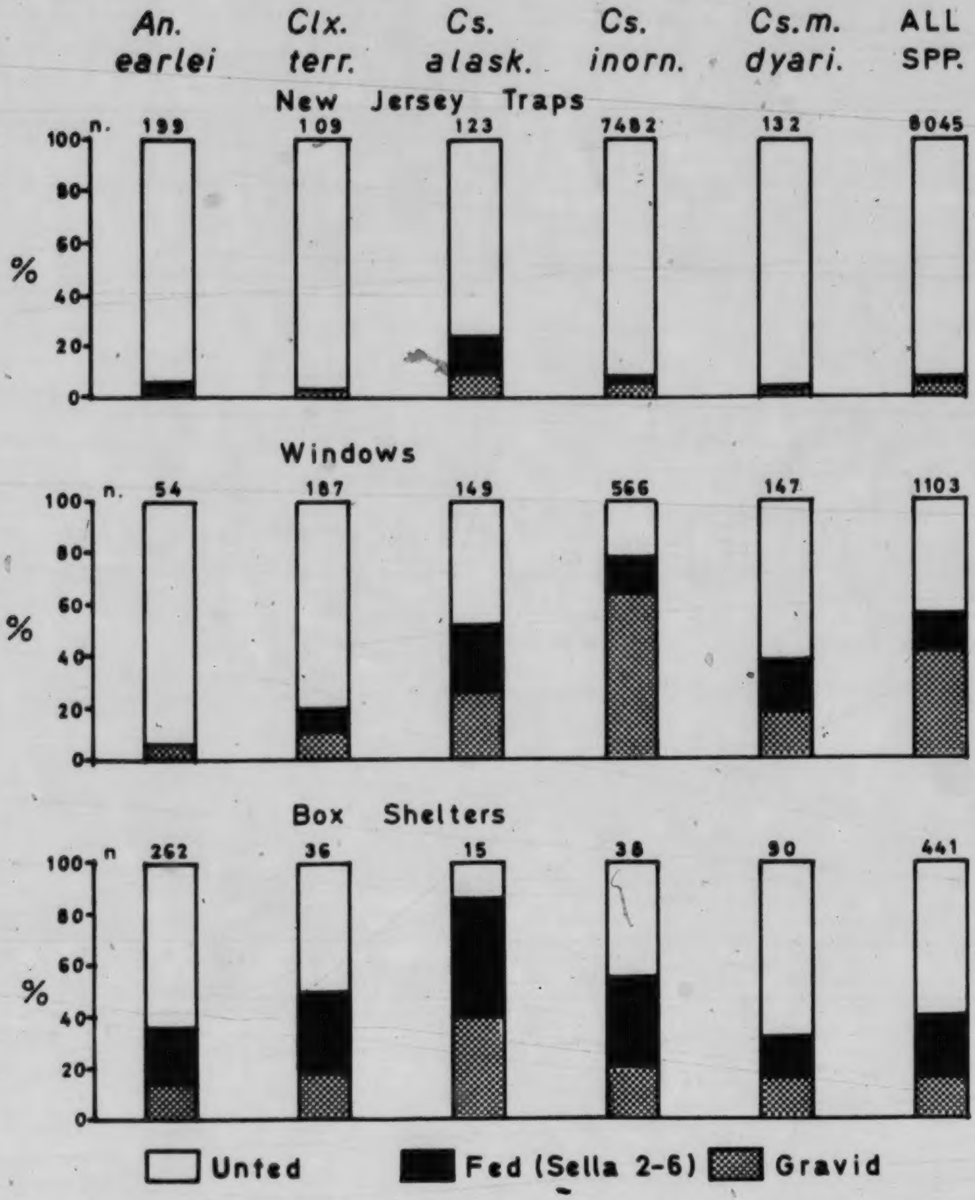


Fig. 6. Relative numbers of unfed, fed and gravid females of five species caught during their gonoactive season by three methods.

17 *Culiseta alaskaensis* and 2 *Anopheles earlei* were counted on the outside of the trap, but only 9 *Cs. alaskaensis* were recovered from it next morning. In August 1974, six one-hour catches were made simultaneously with the trap and from a calf tethered outside, in the first hour after sunset. A heifer and a steer alternated as bait inside and outside. The Williams' Mean catch of *Culiseta inornata* from the trap was less than half what it was outside (Table 4), though the results for all species combined put the trap and the outside catch about equal, and more *Aedes vexans* were taken in the trap. Most of the mosquitoes in the trap were blood fed, 3503 out of 4076, or 86%.

When the Bellamy-Reeves traps were baited with dry ice, 262 mosquitoes were taken in 26 trap-nights, but they included only 1 *An. earlei*, 1 *Cs. alaskaensis* and 1 *Cs. inornata*. When quail were the bait, 243 mosquitoes were taken including 60 *Culiseta morsitans dyari* and 2 *Cs. alaskaensis*. Few of the mosquitoes were engorged, only 8% of the *Cs. m. dyari* and 29% of all species. From mid-July to late August several hundred Ceratopogonidae, (possibly *Culicoides unicolor* Coq.) were found each morning in the two traps in the aspen stand and the spruce bog. More may have been in the traps since they could pass easily through the screen cones. Most of them were blood-fed.

3.1.3. Flying

Two points should be borne in mind when interpreting the results from the New Jersey traps. Firstly, the collecting efficiency

Table 4. Results of simultaneous catches in a calf-baited trap and from a tethered calf outside, in the first hour after sunset, George Lake, 1974.

Date	Bait Calf Inside	*Culiseta inornata* Inside	*Culiseta inornata* Outside	*Aedes vexans* Inside	*Aedes vexans* Outside	All species Inside	All species Outside	Total
14/viii	Heifer	167	314	221	68	287	399	1456
20/viii	Steer	0	14	17	33	21	52	137
21/viii	Heifer	3	11	127	178	151	198	668
26/viii	Steer	0	0	14	6	16	8	44
27/viii	Heifer	0	0	28	18	33	18	97
3/ix	Steer	0	0	87	82	96	86	351
Total		170	339	494	385	604	761	2753
Williams' Mean, M_W		1.8	5.0	49	38	59	60	

of these traps varies with the lunar cycle, being highest at new moon and lowest at full moon (Bidlingmayer, 1974). Secondly, on hot dry days some mosquitoes were unfit for dissection when the trap was emptied. The freshest were dissected and if these were the ones that entered last then there may have been a bias towards that part of the population that flew late in the night. Bidlingmayer (1974) found no difference between the flight periodicities of nulliparous and parous mosquitoes in Florida. The New Jersey trap modified for live catches caught few mosquitoes, but they survived well.

The Malaise trap caught only 136 mosquitoes in nearly 5 months of continuous operation. Poor siting may have been responsible since Graham (1969a) found that the Malaise compared well with the New Jersey and other traps at George Lake.

Only 58 *Anopheles*, *Culex* and *Culiseta* were taken in the truck trap, most of them in the second hour after sunset. Some of the captures of *Aedes* were enormous and subsamples only were taken. Of the 617 females collected, 12 (1.9%) were blood fed, 53 (8.6 %) gravid, and the rest unfed. The trap also caught large numbers of other Diptera, particularly Chironomidae, and Staphylinidae (Coleoptera).

3.1.4. Collections from flowers

Of the 577 mosquitoes collected, about one half were collected from *Tanacetum*(Tansy) and a quarter from *Solidago* (Goldenrod), but the figures (Table 5) may exaggerate the importance of these plants. Most searches were in September, and more time in that month

Table 5. Mosquitoes collected from flowers, George Lake, 1973-75.

Mosquito sp.		Host plant (for full names see Fig. 4.5)											
		Achillea	*Arnica*	*Erigeron*	*Solidago*	*Tanacetum*	*Taraxacum*	*Melilotus*	*Epilobium*	*Galium*	*Heracleum*	TOTAL	% (both sexes)
Anopheles earlei	F	0	0	0	0	0	0	0	0	0	1	1	20.1
	M	0	0	0	7	107	0	0	0	0	1	115	
Culex territans	M	1	0	0	3	42	0	0	0	0	0	46	8.0
Culiseta alaskaensis	F	1	1	0	0	0	0	0	0	0	0	2	2.6
	M	1	3	0	5	3	0	0	1	0	0	13	
Culiseta inornata	F	0	0	0	10	8	0	0	0	0	0	18	16.1
	M	0	0	0	18	57	0	0	0	0	0	75	
Culiseta m. dyari	F	0	0	0	0	1	0	0	0	0	0	1	1.0
	M	0	0	0	1	4	0	0	0	0	0	5	
Culiseta s.minnesotae	F	0	0	0	1	0	0	0	0	0	0	1	4.0
	M	0	0	0	5	17	0	0	0	0	0	22	
Black-legged *Aedes*	F	0	0	0	4	1	0	0	0	0	1	6	2.4
	M	0	0	0	2	0	0	0	0	0	6	8	
Aedes dorsalis	F	0	0	0	2	1	0	0	0	0	0	3	0.5
Aedes excrucians	F	0	0	0	0	1	0	0	0	0	0	1	0.2
Aedes fitchii	F	0	0	0	2	6	0	0	0	0	0	8	1.4
Aedes spencerii	F	0	3	0	12	7	1	1	0	0	1	25	6.9
	M	0	0	1	5	5	0	0	1	0	3	15	
Aedes vexans	F	3	5	0	31	7	0	4	6	2	34	92	31.7
	M	9	2	2	19	16	0	5	5	8	25	91	
Aedes spp. indet.	F	0	2	0	2	10	0	0	2	0	0	16	5.0
	M	1	1	0	4	6	0	0	0	0	1	13	
TOTAL		16	17	3	133	299	1	10	15	10	73	577	99.9
%		2.8	2.9	0.5	23.0	51.8	0.2	1.7	2.6	1.7	12.6	99.8	

was spent searching them than other plants. *Heracleum* (cow parsnip) was a good source of mosquitoes during its rather short flowering period. The flowering dates of the following plants are shown in Fig. 7: *Achillea millefolium* L. (yarrow),*Arnica chamissonis* Less., *Aster spp.*, *Crepis tectorum* L. (annual hawksbeard), *Erigeron glabellus* Nutt. (smooth fleabane), *Petasites sagittatus* (Pursh.) (arrow-leaved coltsfoot), *Solidago spp.* (goldenrod), *Sonchus uliginosus* Bich (perennial sow thistle), *Tanacetum vulgare* L. (tansy), *Taraxacum officionale* Weber (dandelion), *Medicago sativa* L. (alfalfa), *Melilotus alba* L. and *M. officionalis* (L.) (white and yellow sweet clovers), *Trifolium pratense* L. and *T. repens* L. (red and white clovers), *Epilobium angustifolium* L. (fireweed, willow herb), *Ranunculus acris* L. (tall buttercup), *Rosa acicularis* Lindl. (prickly rose), *Galium boreale* L. (northern bedstraw), and *Heracleum lanatum* Michx. (cow parsnip). The absence of lines for some species in some years does not mean that they did not flower, but simply that the dates were not recorded. Mosquitoes were seen on flowers from late May to late September, (Table 6). Of the 174 females collected, 33 were blood-fed (18.5%) and 9 gravid (5.2%), and there would probably have been more if observations had been made earlier in the year.

A damsel bug (Heteroptera: Nabidae) was seen sucking a *Culex territans* male on *Tanacetum*, 1 hour after sunset on 25/ix/74. The most productive search was one of the latest in the year, from 45 to 75 minutes after sunset on 25/ix/74, the temperature an unusually high 16 C, when 150 *Tanacetum* stems yielded 8 female and 156 male mosquitoes.

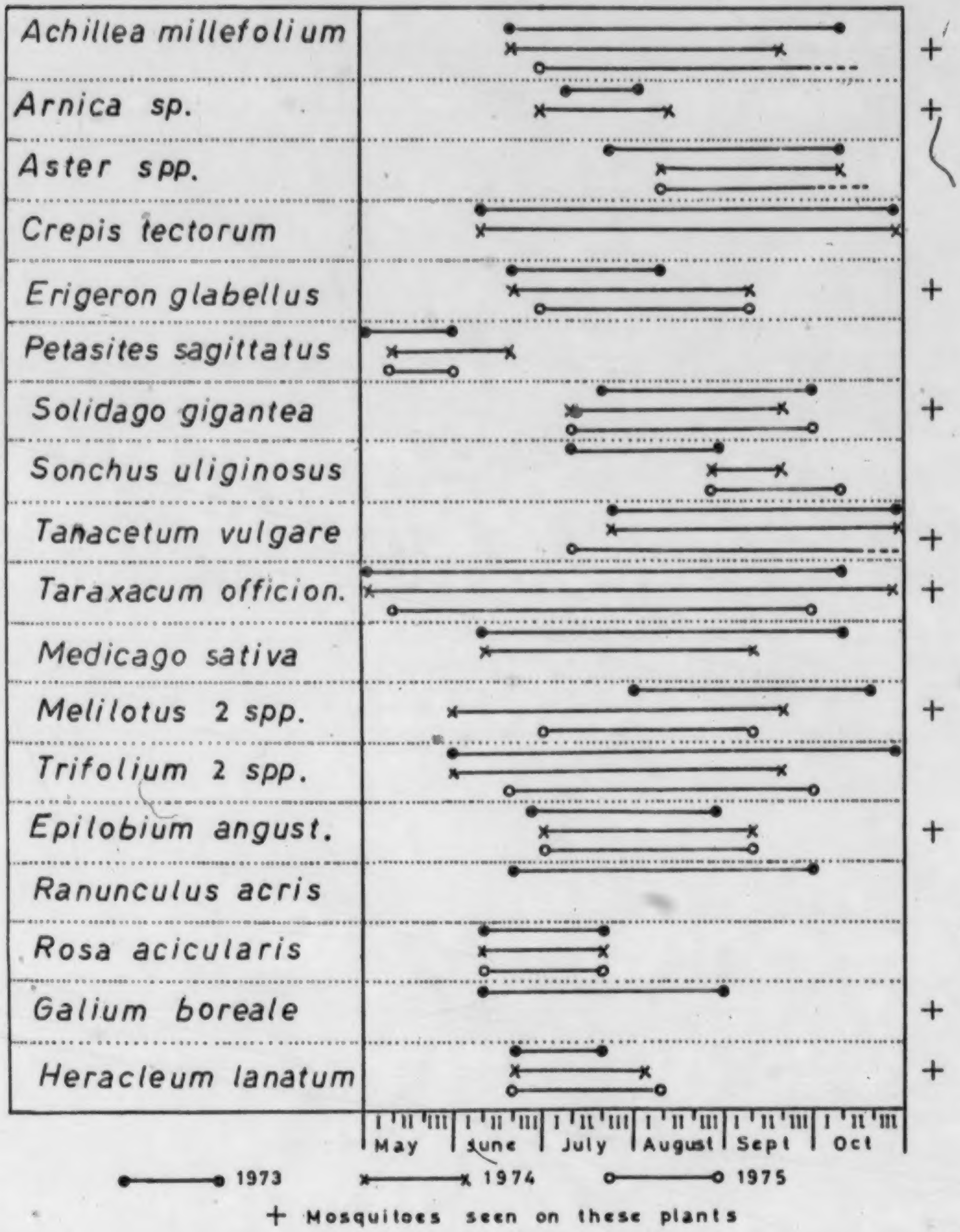


Fig. 7. Flowering periods of some plants at George Lake, 1973-75. Full names given in text.

Table 6. Seasonal distribution and abdominal condition of mosquitoes collected from flowers.

		May		July			August			September			
		III	I	II	III	I	II	III	I	II	III	TOTAL	%
Anopheles earlei	G*	0	1	0	0	0	0	0	0	0	0	1	
	M	0	0	1	0	0	0	6	6	33	69	115	
Culex territans	M	0	0	1	0	0	0	0	4	0	41	46	
Culiseta alaskaensis	UF	0	0	2	0	0	0	0	0	0	0	2	
	M	0	0	5	0	0	1	2	5	0	0	13	
Culiseta inornata	UF	0	0	0	0	1	0	2	1	8	0	12	
	F	0	0	0	0	1	0	2	0	0	0	3	
	G	0	0	0	0	1	0	2	0	0	0	3	
	M	0	0	0	0	0	0	5	31	13	26	75	
Culiseta s. minnesotae	UF	0	0	0	0	0	0	0	1	0	0	1	
	M	0	0	0	0	0	1	0	6	1	14	22	
Culiseta m. dyari	UF	0	0	0	0	0	1	0	0	0	0	1	
	M	0	0	0	0	0	1	4	0	0	0	5	
Black-legged *Aedes*	UF	0	1	0	0	3	0	1	0	0	0	5	
	F	0	0	1	0	0	0	0	0	0	0	1	
	M	0	1	5	0	1	0	1	0	0	0	8	
Aedes dorsalis	F	0	0	0	0	0	0	0	0	1	0	1	
	G	0	0	0	0	0	0	0	2	0	0	2	
Aedes excrucians	F	0	0	0	0	0	0	0	0	0	1	1	
Aedes fitchii	UF	0	0	0	0	2	0	0	0	0	1	3	
	F	0	0	0	0	1	0	0	1	1	2	5	
Aedes spencerii	UF	0	1	1	1	3	0	0	0	11	0	17	
	F	1	0	5	0	2	0	0	0	0	0	8	
	M	0	3	1	1	0	0	0	0	10	0	15	
Aedes vexans	UF	0	3	44	2	3	0	6	8	7	2	75	
	F	0	0	4	1	4	0	0	0	3	2	14	
	G	0	0	0	0	1	0	0	2	0	0	3	
	M	0	8	40	12	4	0	12	3	6	6	91	
Aedes spp. indet.	UF	0	0	4	0	0	2	8	2	0	0	16	
	M	0	0	4	0	0	0	8	1	0	0	13	
All species	UF	0	5	51	3	12	2	18	12	26	3	132	76.3
	F	1	0	10	1	8	0	2	1	5	5	33	18.5
	G	0	1	0	0	2	0	2	4	0	0	9	5.2
	M	0	12	57	13	5	2	35	60	63	156	403	
TOTAL		1	18	118	17	27	4	57	77	94	164	577	

* UF = Unfed, F = Blood fed, G = Gravid females; M = Males.

3.1.5. Resting

Resting *Culex* and *Culiseta* adults flew out of the box shelters at the slightest disturbance and did not come back. The *Anopheles earlei* were much less easily disturbed, and some which did fly out hovered at the entrance, flew back in and settled. Sometimes *Aedes* females which had been buzzing round my head as I knelt down to empty the boxes would form a small, compact swarm round the entrance for a few seconds after I stood up.

3.2. Dissections

Information on insemination, crop contents and the lengths of ovaries, germaria and follicles were recorded only in 1974 and 1975. The other information was recorded for dissections in all years.

In all species the follicle lengths and F:G ratios increased by similar amounts between stages I and IIb, because the germaria showed little increase, (Table 7). The F:G ratios were chosen for presentation because of their independence of the units of measurement, and for ease of comparison between species. The ovary lengths showed similar patterns to the F:G ratios and will not be presented.

3.3. Blood meal identifications

One hundred and ninety-one of the 250 blood meals reacted (76 %). Most of the identified meals were from bovines. Individual species patterns will be discussed in later sections. The "unidentified mammal" and "unidentified bird" results were not necessarily from feeds on hosts for which there were no antisera, and most were partly

Table 7. Germarium and follicle lengths and F:G ratios of females at various stages of follicle development. Means ± standard deviations in µm.

			Follicle Stage			Relative increase
	N1	N2	I	IIa	IIb	IIb:I
Anopheles earlei						
Number of females	2	9	469	70	69	
Germarium length	62 ± 6	31 ± 5	41 ± 4	40 ± 6	42 ± 3	1.02
Follicle length	-	43 ± 6	76 ± 19	106 ± 11	119 ± 4	1.56
F:G ratio	-	1.41	1.88	2.74	2.86	1.52
Culex territans						
Number of females	1	12	436	44	20	
Germarium length	41	31 ± 5	40 ± 4	38 ± 6	39 ± 5	0.98
Follicle length	-	39 ± 6	57 ± 11	98 ± 13	115 ± 9	2.02
F:G ratio	-	1.27	1.41	2.62	2.98	2.11
Culiseta alaskaensis						
Number of females	58	39	307	73	55	
Germarium length	71 ± 15	45 ± 9	45 ± 6	45 ± 6	48 ± 6	1.07
Follicle length	-	58 ± 5	94 ± 21	118 ± 15	136 ± 15	1.45
F:G ratio	-	1.30	2.09	2.61	2.87	1.37
Culiseta inornata						
Number of females	7	42	685	166	137	
Germarium length	46 ± 4	26 ± 7	39 ± 5	40 ± 5	42 ± 3	1.08
Follicle length	-	39 ± 7	66 ± 21	110 ± 13	132 ± 16	2.00
F:G ratio	-	1.35	1.72	2.73	3.14	1.82
Culiseta m. dyari						
Number of females	1	12	124	30	69	
Germarium length	58	29 ± 8	40 ± 6	42 ± 5	42 ± 2	1.05
Follicle length	-	45 ± 3	80 ± 18	108 ± 10	131 ± 40	1.64
F:G ratio	-	1.63	2.04	2.61	3.15	1.55

digested meals that reacted too weakly for detection with the specific antisera, (Dr. J. D. Edman, in litt.).

The majority of *Culiseta inornata*, *Cs. alaskaensis* and *Aedes fitchii* had fed on bovines, not only at George Lake but also at the University of Alberta, Edmonton (Table 8a). There were a few cattle at a zoo 2.4 km west of campus across the river valley, and more at the University farm 3.2 km south, and a meat packing plant 3.2 km east. If the mosquitoes had fed on the nearest of these cattle they would have flown twice as far after their meals as the *Aedes* in Florida described by Edman and Bidlingmayer (1969). About one third of the bovine positives collected in Edmonton were in Sella stage 2, which suggests that they had fed the night before, (Table 8b).

3.4. Nectar tests

The presence of nectar was confirmed in 293 out of 554 mosquitoes collected between early June and early August (Table 9). The proportion of nectar positives was lower among females collected from bait than among those collected from other sites. These results and the collections from flowers show that some of the females fed on flowers before digesting blood and fed on blood before digesting nectar. The low number of nectar-positive *Culiseta inornata* (22 %) in early August may have been due to the low room temperature when the test was conducted, 10 - 12 C.

3.5. Virus tests

No arboviruses were isolated from any of the 53 pools of mosquitoes submitted (8 *Culiseta inornata*, 23 *Aedes vexans*, and the

Table 8. (a). Numbers of blood-fed mosquitoes that had fed on bovines, collected at George Lake and Edmonton (Positive identifications only.)
(Precipitin tests by Florida Medical Entomology Laboratory).

	George Lake				U. of A. Campus			
	Bovines		Other hosts		Bovines		Other hosts	
	No.	%	No.	%	No.	%	No.	%
Culiseta alaskaensis	9	100	0	0	8	89	1	11
Culiseta inornata	35	94	2	6	45	90	5	10
Aedes fitchii	3	100	0	0	5	56	4	44
All 3 species	47	96	2	4	58	85	10	15

(b). Proportions of bovine-feds collected on U. of A. campus in different stages of Sella.

	Sella Stage				
	2	3	4	5	Total
Culiseta alaskaensis	2	0	6	0	8
Culiseta inornata	17	17	11	0	45
Aedes fitchii	2	1	1	1	5
Total	21	18	18	1	58
%	36	31	31	2	

Table 9. Anthrone tests for nectar in mosquitoes, 1973-74. All mosquitoes were unfed females unless otherwise indicated.

		June						July						August		Total		
		I		II		III		I		II		III		I				
		No. test	No. +ve	No. test	No. +ve	No. test	No. +ve	No. test	No. +ve	No. test	No. +ve	No. test	No. +ve	No. test	No. +ve	No. test	No. +ve	% +ve
At bait																		
Culiseta alaskaensis		12	6													12	6	50
Culiseta inornata		5	2							72	45	139	64	96	21	312	132	42
Aedes spp.		107	82	55	25	17	9									179	116	65
Total		124	90	55	25	17	9			72	45	139	64	96	21	503	254	
Percent positive			72		45		53				62		46		22		50	
At other sites																		
Culiseta alaskaensis	F							10	9							10	9	90
	M							41	31							41	31	76
Culiseta inornata								29	24							29	24	83
Anopheles earlei								1	1							1	1	
Culex territans								1	1							1	1	
Aedes spp.						10	4									10	4	40
Total (Females)						10	4	41	35							51	39	
Percent positive							40		85								76	
Combined Total																554	293 53	

remainder representing 10 other species), nor from any of the quail blood samples.

3.6. Collection and identification of larvae

When all the collections from the lakeside pond are added together, (Table 10), there were fewest first instar larvae and most fourth instar larvae: since in any population there is bound to be mortality in each instar, the sampling method must have been far less efficient for the earlier than for the later instars.

All *Culex* were presumed to be *territans* and all *Anopheles* to be *earlei*. *Culiseta* larvae with short, thick siphons were presumed to be either *Cs. alaskaensis* or *Cs. inornata* since these were the only two species identifiable among the fourth instar larvae or reared from pupae. The head capsule widths alone were not sufficient to separate the two species, because of some overlap between the ranges for second, third and fourth instar *Cs. alaskaensis* and third and fourth instar *Cs. inornata* (Table 11). In all instars the antennal spicules of *Cs. inornata* were sparser and smaller than those of *Cs. alaskaensis*, (Steward and McWade, 1961), and this additional character sufficed to separate the species. The other *Culiseta* larvae collected from the pond were all *Cs. s. minnesotae* except for one *Cs. m. dyari* in August, 1973.

3.7. *Anopheles earlei*

Females were taken at bait from late April to early June and from late June to mid-August, but the greatest number ever taken was 11 in one hour in late May, 1973, and the mean numbers never

Table 10. Total numbers of larvae and pupae collected in lakeside pond, George Lake, 1973-75.

	Instar					
	I	II	III	IV	Pupae	Total
Anopheles earlei	71	166	160	282	86	765
Culex territans	79	309	634	783	122	1927
Culiseta alaskaensis	64	117	129	57	58	425
Culiseta inornata	129	191	131	193	188	832
Culiseta s. minnesotae(a)	0	1	9	18	19	47
TOTAL	343	784	1063	1333	473	3996
Percent	8.6	19.6	26.6	33.4	11.8	
Ratio to no. of I instars	1.00	2.28	3.10	3.88	1.38	

(a) includes one larva of *Culiseta morsitans dyari*, instar unknown.

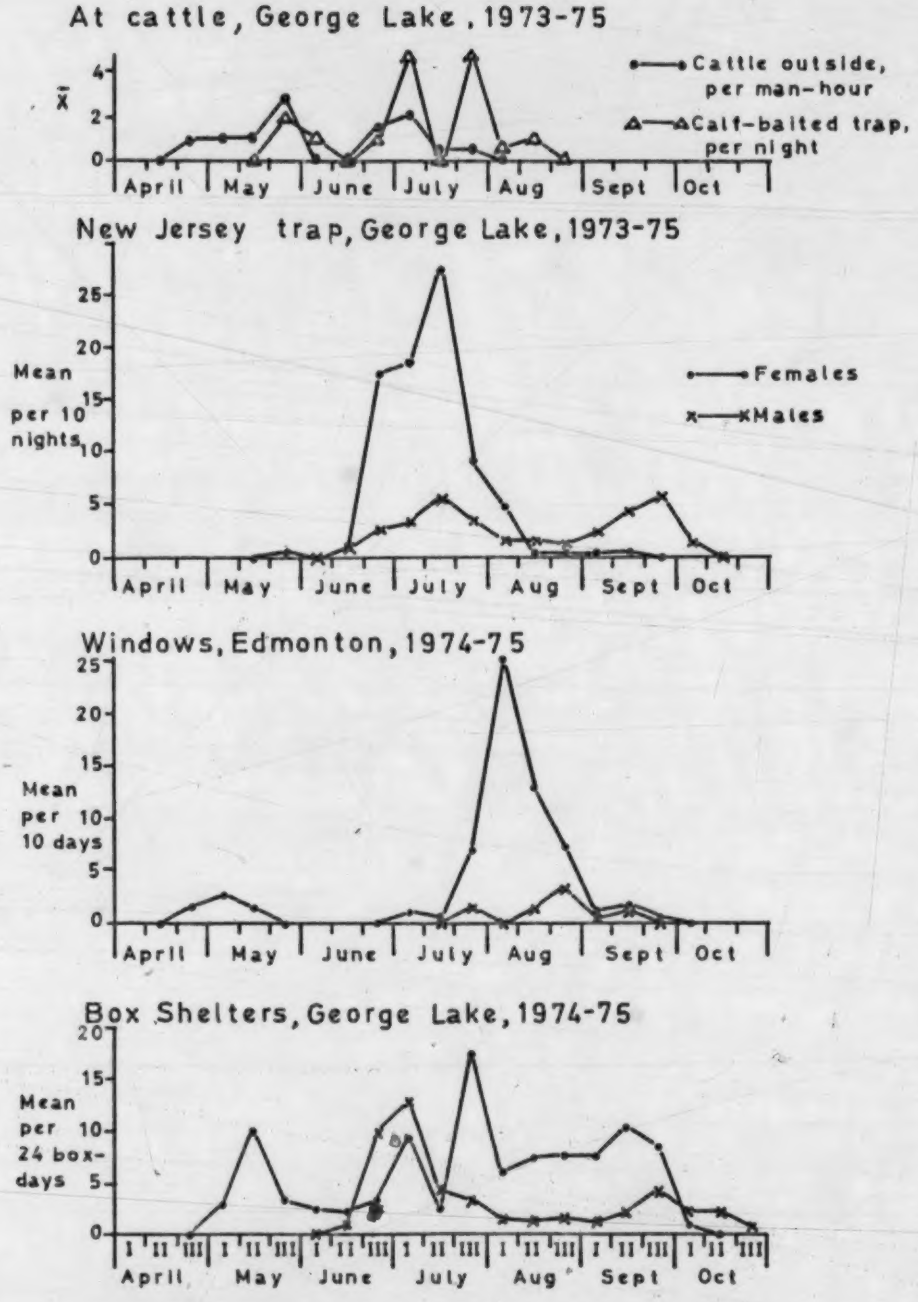
Table 11. Head capsule widths (mm) of larvae reared in the laboratory at 20 C, from mothers caught in the Edmonton region.

	Instar			
	I	II	III	IV
Anopheles earlei				
Mean	0.20	0.30	0.50	0.80
Range	0.20-0.22	0.28-0.32	0.42-0.52	0.72-0.88
Standard deviation	0.01	0.01	0.03	0.04
Number measured	15	13	24	23
Culex territans				
Mean	0.30	0.50	0.84	1.18
Range	0.28-0.32	0.42-0.52	0.77-0.90	1.15-1.28
Standard deviation	0.02	0.03	0.03	0.06
Number measured	20	20	20	14
Culex tarsalis				
Mean	0.29	0.47	0.84	1.30
Range	0.25-0.30	0.42-0.52	0.75-0.90	1.18-1.35
Standard deviation	0.02	0.04	0.04	0.04
Number measured	20	20	20	20
Culiseta alaskaensis				
Mean	0.46	0.74	1.12	1.62
Range	0.42-0.48	0.65-0.80	1.02-1.18	1.50-1.78
Standard deviation	0.02	0.04	0.04	0.08
Number measured	20	20	20	20
Culiseta inornata				
Mean	0.32	0.53	0.89	1.35
Range	0.30-0.35	0.50-0.58	0.80-0.98	1.18-1.52
Standard deviation	0.03	0.05	0.09	0.20
Number measured	20	18	20	20

exceeded 4.4 per hour from cattle outside and 5 per night in the trap (Fig. 8). Humans were sometimes attacked in spring during the day. The numbers of females at other sites show 3 peaks. The first, in May, represented the emergence of the overwintered females. The second occurred in mid-July, late July or early August according to the method of collection. The appearance of males, teneral and nulliparous females (see below), suggest that the second peak represented the appearance of the first summer generation. A third peak of females in mid-September in the box shelters may have represented the emergence of the second summer generation. Collections of males in the New Jersey trap and in the boxes reached a peak in late September, and a few were taken in the boxes in late October the last time they were searched.

The first gravids and par were taken in early May, and in each succeeding decade through to late August (Fig. 9). The presence of tenerals indicates the emergence of females from late June through to mid-September. Of special interest is the decrease in the proportion parous from early to late June, which indicates that at least some of the first summer generation took blood meals.

The first larvae were found in the lakeside pond in late May, (Fig. 10), but must have been present before this since the first collection in 1973 had larvae in all four instars, and the first pupae were taken in mid-June. There was a second generation in 1974 but not in 1973 or 1975. Larvae were found in several other permanent pools in the area (I, II, III, IV, V and VII in Fig. 2).



At cattle, George Lake, 1973-75

New Jersey trap, George Lake, 1973-75

Windows, Edmonton, 1974-75

Box Shelters, George Lake, 1974-75

Fig. 8. Seasonal abundance of *Anopheles earlei*.

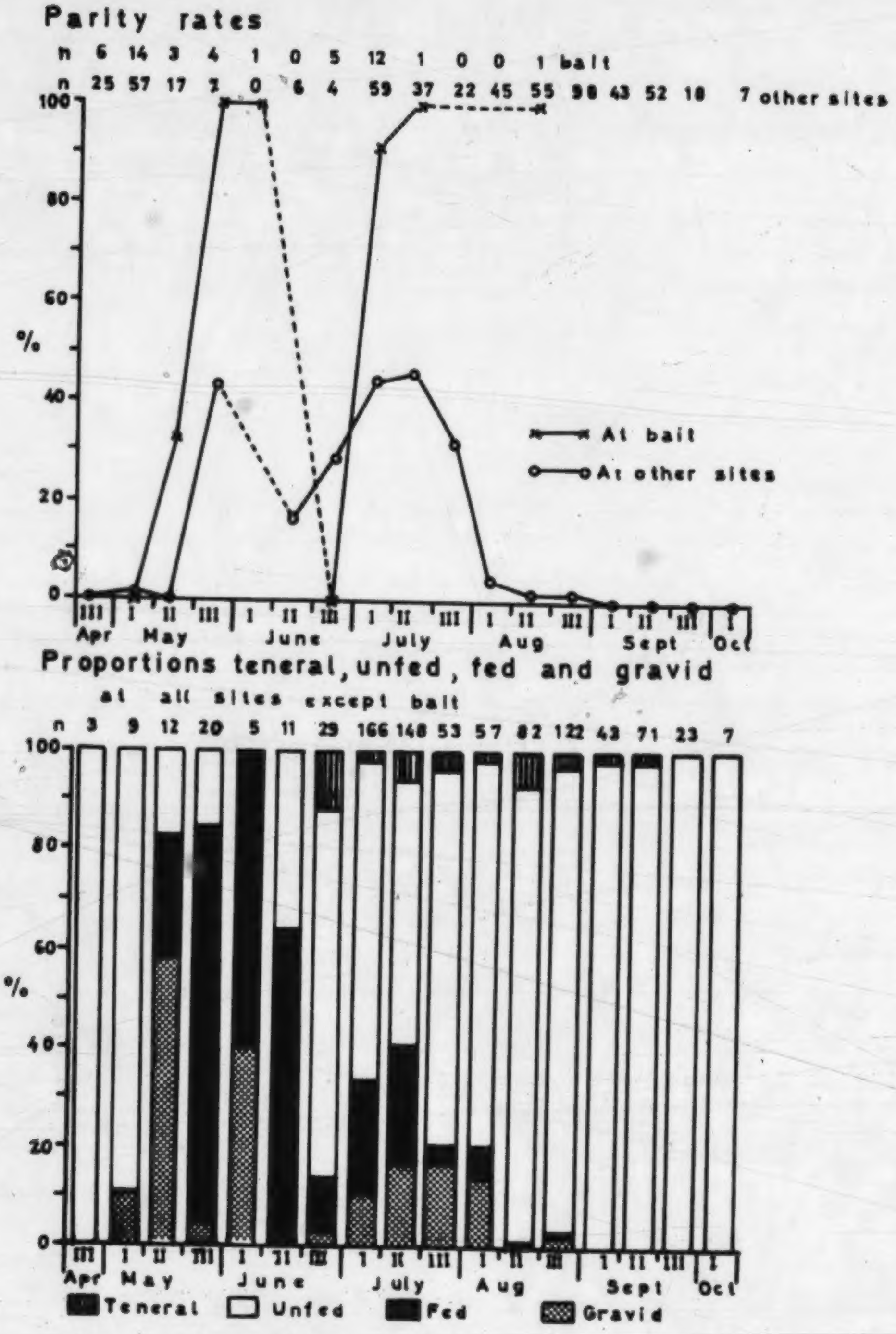


Fig. 9. Seasonal changes in fed, gravid and parous rates of *An. earlei*.

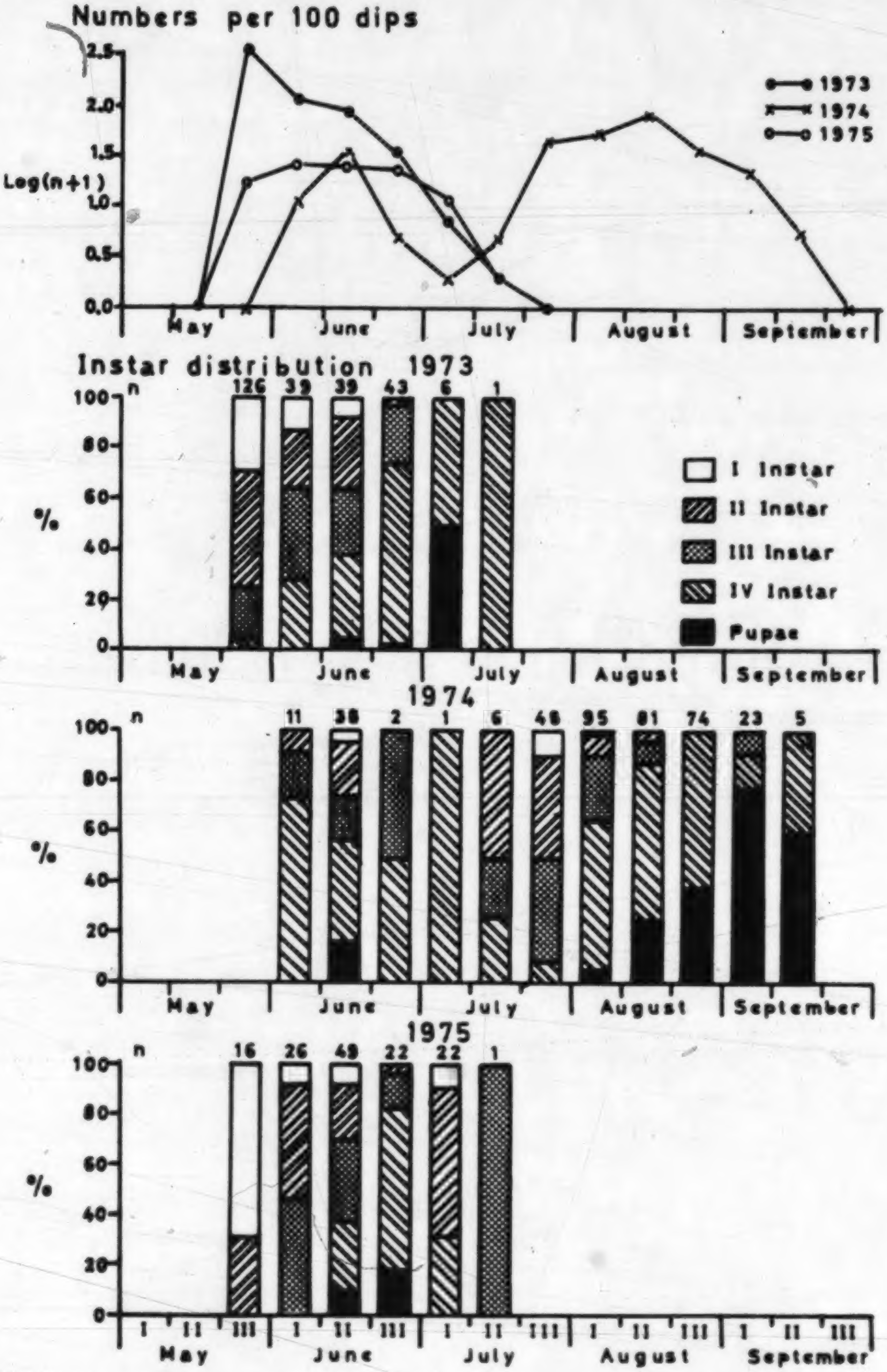


Fig. 10. Seasonal abundance and instar distribution of *An. earlei* larvae and pupae in the lakeside pond.

Of 59 blood meals tested only 32 reacted. Thirteen of them were from rabbit (41%), 6 bovine (19%), 1 cat (3%) and there were 12 "unidentified mammal" (37%), all but one of them in Sella stages 4 or 5. All the females that had fed on rabbits were in a pig house and a sheep house on the east side of the farmyard. Two domestic rabbits were kept in a shed on the other side of the yard, and no wild hares were ever seen. Since larger and more numerous mammals were present, the results suggest a preference for rabbits. The closely-related *An. freeborni* in California seems to prefer rabbits where the choice exists (Tempelis, 1975).

Fifty-five females laid or contained a mean of 135.9 eggs (range 65 - 267, standard deviation 40.4). Eight of 83 pars (9.6%) had retained eggs, 4 with 1, 3 with 2 and 1 with 3. there was no clear seasonal trend in the numbers of eggs produced or retained.

Eighteen tenerals were examined, most of them were from boxes close to the lakeside pond, where they probably came from. Nine still had meconium, 16 were uninseminated and 14 had no visible fatbody development, but in 10 of 16 the follicles had reached stage I.

The follicles of females at bait were larger and more advanced than those of females at other sites, (Fig. 11). Most of the nullipars at bait had F:G ratios greater than 2.0 and all had follicles in stage II. The pars at bait all had F:G ratios greater than 2.0 but 20% had follicles in stage I.

The mean F:G ratio of all the nullipars collected was at its highest in early May, when the females were emerging from their

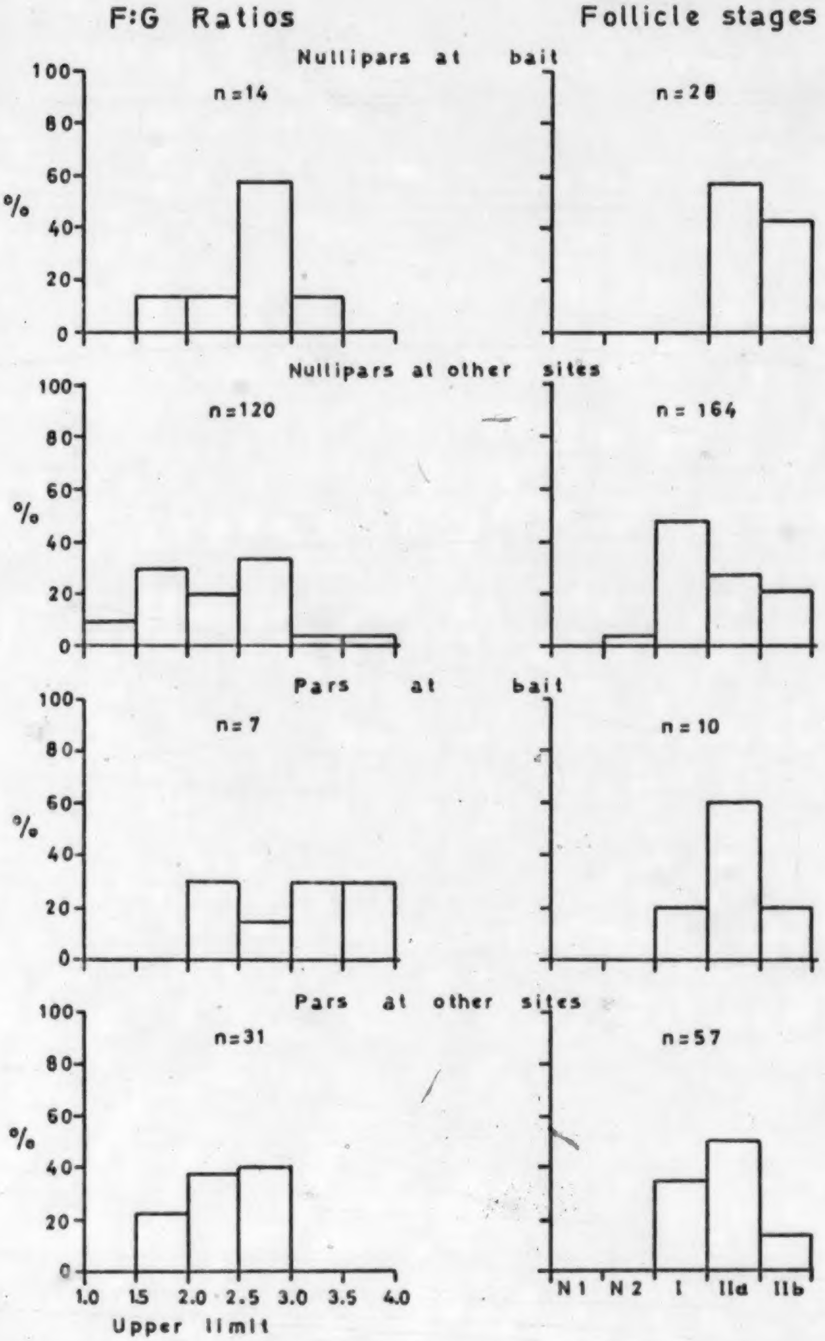


Fig. 11. Distribution of F:G ratios and follicle stages of *An. earlei* in relation to parity and biting activity.

overwintering sites, and at its lowest in late October when they were going into the overwintering sites, (Fig. 12). The period from late July to late October, when the mean F:G ratio was less than 2.0 was the period when no nullipars were taken at bait and very few feds and gravids were collected anywhere, (Fig. 9), although weather conditions still allowed feeding by *Aedes spp*. If we set an upper limit of 2.0 for the F:G ratios of females in diapause, some difficulties follow. Firstly, some nullipars taken at bait and presumably gonoactive had F:G ratios of less than 2.0. Secondly, the pattern of distribution of F:G ratios during summer, (Fig. 13), shows that the majority had F:G ratios of less than 2.0 in July, when nullipars were still being taken at bait, and a few with F:G ratios of more than 2.0 even in late September, when all blood feeding appeared to have ceased. Some of the females with small follicles in July could have been early post-tenerals whose follicles were still growing. Thus it seems generally, but not invariably, true that diapausing *A. earlei* may be recognized by an F:G ratio of less than 2.0. From this it would follow that the females collected during winter were no longer in diapause, and some support for this view was provided by the readiness of females collected in January to take blood and then to mature eggs, (see Chapter 4).

Most of the overwintered females in April and May still had abdomens distended with fatbody, (Fig. 14). In July few females had well-developed fatbodies. In August there was a rapid increase in fat nullipars, but not pars, and the abdomens of nearly all the

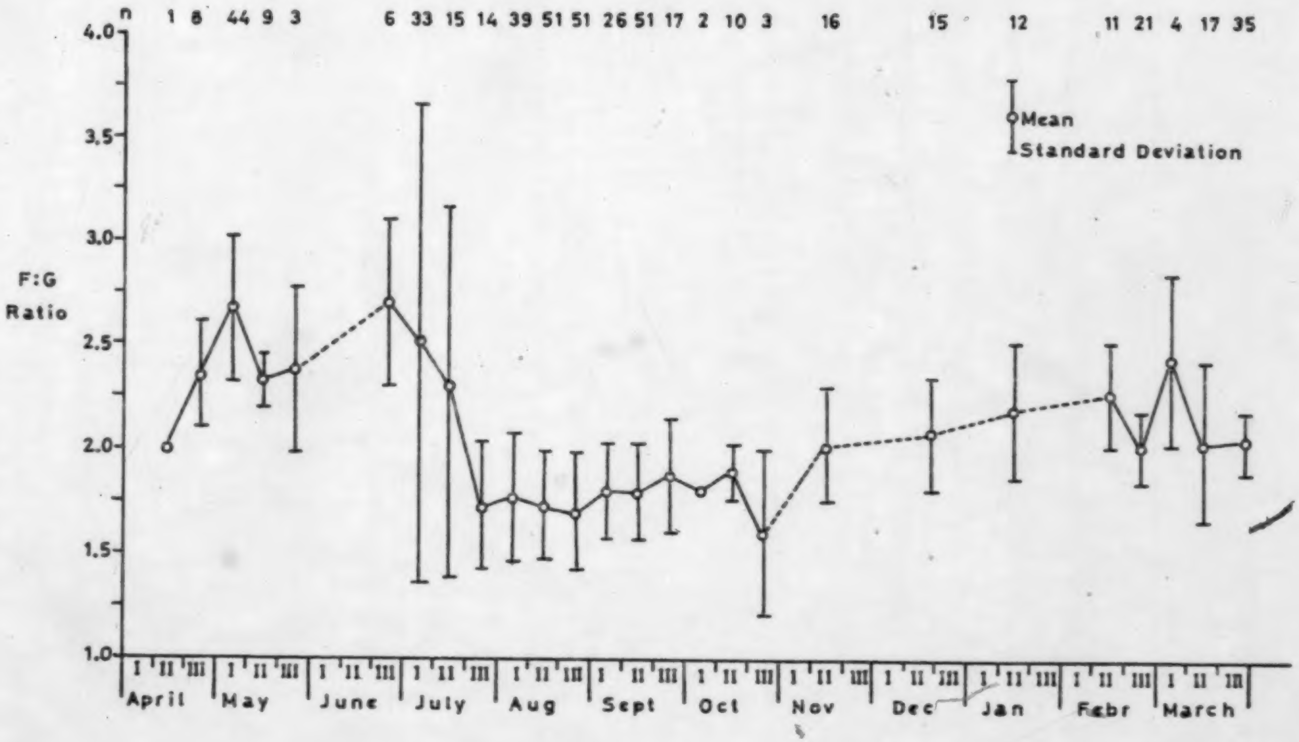


Fig. 12. Mean F:G ratios of nulliparous *An. earlei* throughout the year. At sites other than bait.

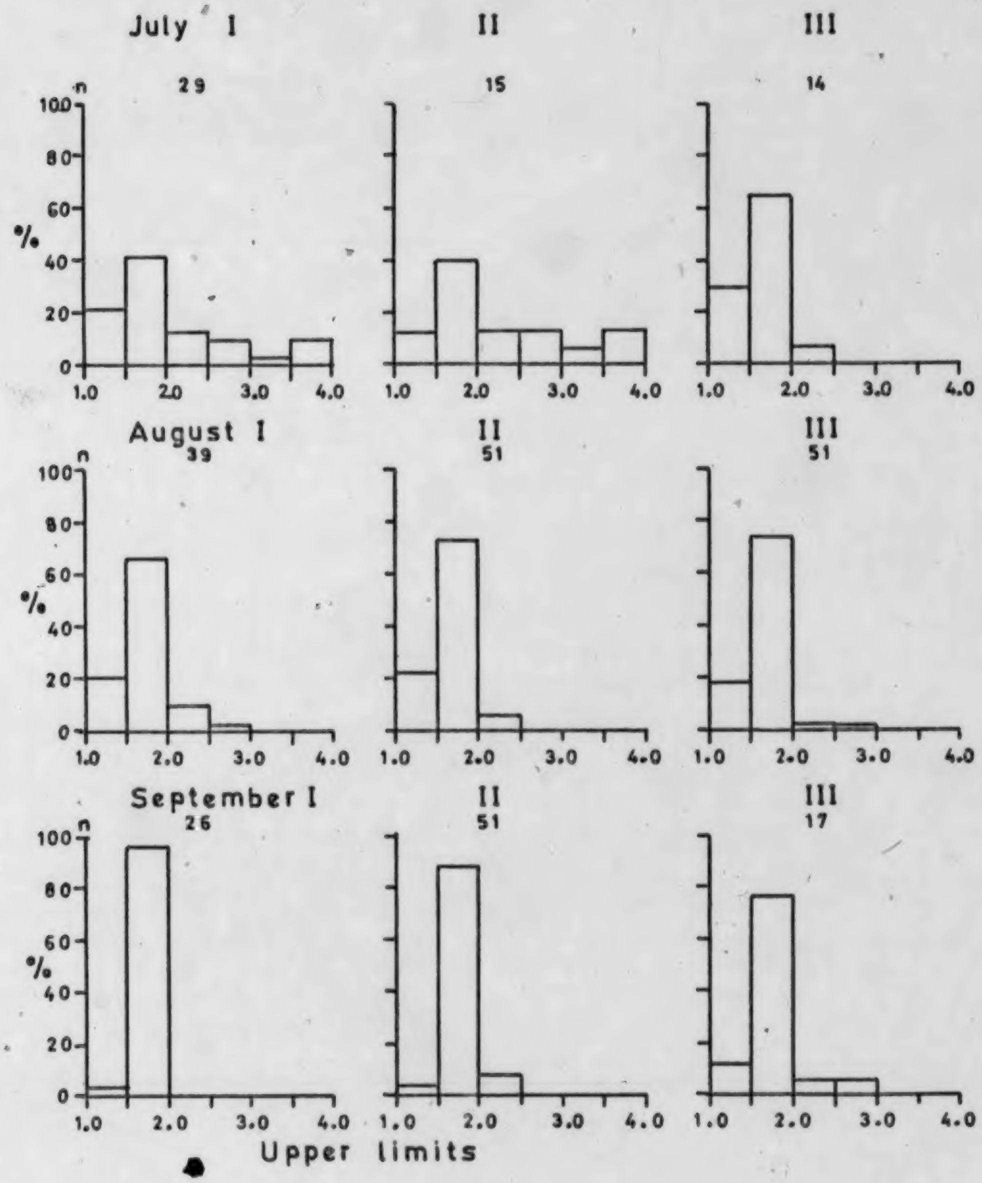


Fig. 13. Distribution of F:G ratios of nulliparous *An. earlei* in July, August and September. At sites other than bait.

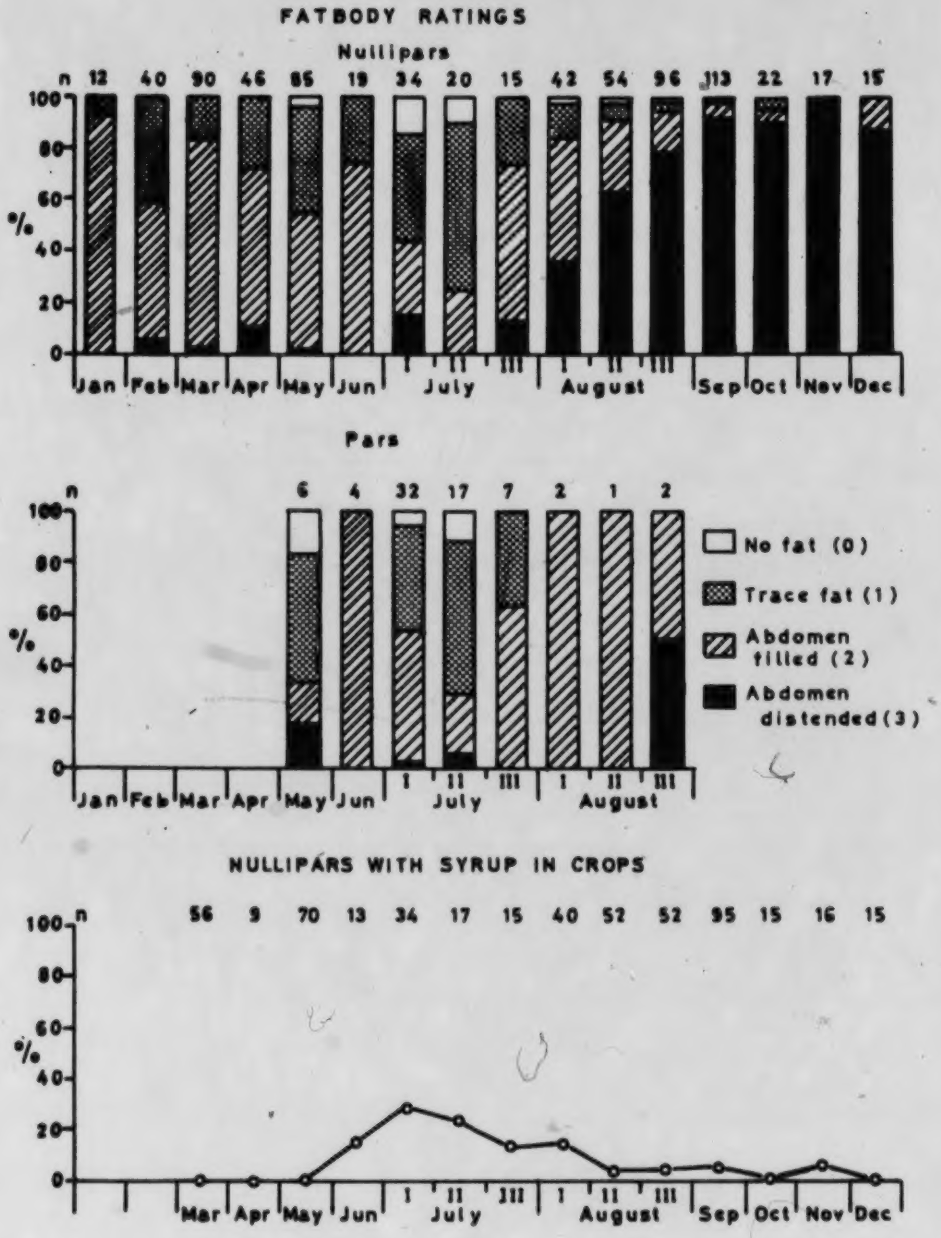


Fig. 14. Seasonal changes in fatbody ratings and crop contents of nulliparous *An. earlei*.

nullipars in hibernation remained distended with fat until December. It was surprising that a population showing such a rapid increase of fat nullipars had so few with syrup in their crops (Fig. 14); a maximum of 29 % had syrup in early July. Nine of 48 pars (19%) had syrup. Three females from the box shelters, 1 on 14/viii/74 and 2 on 27/viii/74, had rod-shaped bacteria-like bodies in the syrup.

On 9/v/76 the beaver lodge nearest the lake (see Fig. 2), was opened by two other students, J. S. Ashe and H. Frania. They saw many mosquitoes inside and brought back 20 *An. earlei* females, 9 of them blood-fed.

I reopened the lodge on 17/v/76, which the beavers had repaired with a plug of loose twigs, branches and mud. The inside was a dome 1.2 m wide, 0.7 m high, and 1.8 m long, with a tunnel out into the pond running another 0.6 m before its roof met the water. The ceilings of the chamber and the tunnel were thickly coated with *An. earlei* females.

Of 1362 *An. earlei* females collected, 828 were apparently unfed, 492 blood-fed (some with very small meals), and 42 gravid, but 3 of 59 "unfeds" dissected had traces of blood in their guts. Allowing for these "cryptic feds", the proportions were 56.7 % unfed, 40.2 % fed and 3.1 % gravid. All 59 dissected were parous, 16 (27 %) had retained eggs, and almost all had fatbody ratings of 1 and follicles in stage II. There were also 3 fed and 3 gravid *Culex territans*, and 116 black-legged *Aedes* (3 fed) which probably all came in after I opened the roof.

This collection does not prove that any of the mosquitoes overwintered in the beaver lodge. All the unfed *An. earlei* were parous, and flying and blood feeding were observed before mid-May in other years. If *An. earlei* females do not only rest in the lodges but regularly feed on beavers, they may be vectors of Tularemia, which often occurs in beavers. The bacillus has been transmitted by *An. maculipennis* in the laboratory, and found in nature in this and 10 other mosquito species in the U.S.S.R., (Sazanova, 1962).

The observations on seasonal distribution are summarised in Fig. 15. The figure does not try to indicate relative abundance since the finding of only one specimen in any of the categories was enough to fill the square. It is difficult to place the time of the onset of diapause from F:G ratios when tenerals are present, or from the disappearance of feds and gravids when pars are present. Consideration of relative abundance would place it some time between mid-July and mid-August. A closer estimate is attempted in Chapter 5.

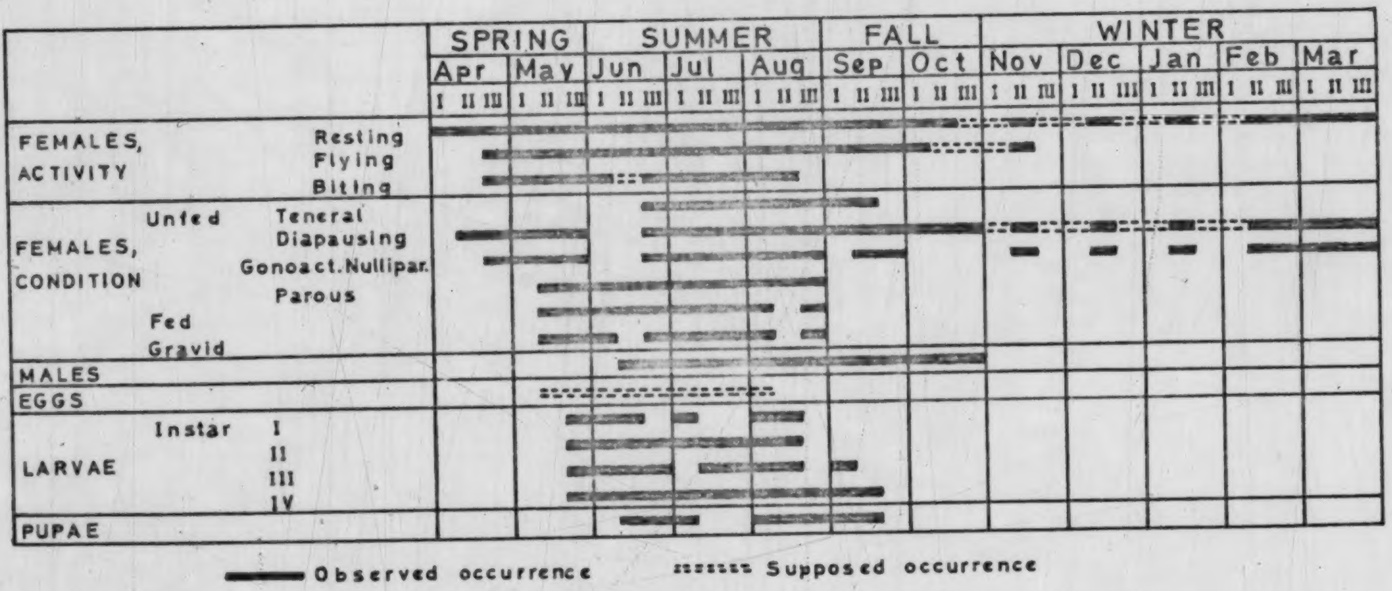


Fig. 15. Summary of seasonal biology of *An. earlei*.

3.8. *Culex tarsalis*

Only 24 females and 10 males were collected between 1972 and 1975. The Bellamy-Reeves trap has taken large numbers of *Clx. tarsalis* (Bellamy and Reeves, 1952), and New Jersey traps have been used to measure population dynamics of this species in Saskatchewan (McLintock and Rempel, 1963) and California (Reeves et al., 1964). Since I tried both traps and collected few *Clx. tarsalis*, it does seem to be rare in the study area.

Females were collected from late July to late October, with the largest numbers in mid-September, (Table 12). Three gravids collected in early and mid-August had a mean of 139 eggs (120, 187, 110). Six nullipars had F:G ratios of 1.75 in August I (1), 1.20 in August III (1), 1.25 in September I (1), and 1.25 in September III (2). If the rest of the nullipars were in diapause by early August, they would play no part in the transmission of arboviruses after the end of July.

Table 12. Summary of collections of *Culex tarsalis* at Edmonton and George Lake, 1972-75.

	Females			Males	Total
	Unfed	Fed	Gravid		
July III	1	1[a]	0	0	2
August I	3	1(b)	3	0	7
II	0	0	1	0	1
III	1	0	0	2	3
September I	2	0	0	2	4
II	5	0	0	3	8
III	3	0	0	1	4
October I	2	0	0	1	3
II	0	0	0	1	1
III	1	0	0	0	1
Total	18	2	4	10	34

(a) Box shelter, George Lake.

(b) Pig house, George Lake.

All others from Edmonton.

3.9. *Culex territans*

Little information was obtained on seasonal abundance because no females were taken at bait and few at any of the other sites, except during the fall. The first peak, in May, indicated the emergence of the females from overwintering sites, (Fig. 16). A second peak, in late June or early July marked the emergence of the first summer generation, and a third peak in September the second summer generation. Why the first summer generation was so poorly represented and the second so well-represented in the window collections is puzzling. A female was swept from vegetation in summer, and it may be that they only rest on windows in spring and fall, when there is no green foliage to rest in. Males were taken from mid-June to late October. The peak collections coincided with those for the females in the New Jersey traps, but the main peak in the window collections was in late August. Both females and males were taken in the last box shelter collections in late October.

The first feds were collected in late April, the first gravids in early May, and the last feds and gravids in late July, (Fig. 17). Few tenerals were collected, the earliest in mid-July, but the presence of pupae in mid-June, (Fig. 18), suggests that the first summer generation began to emerge in June. The decrease in the percentage of nullipars and the increase in the percentage of feds and gravids, although slight, suggest that at least some of the first summer generation were gonoactive and took blood meals. The rapid buildup of diapausing females, which reached 50% of the nullipars by late

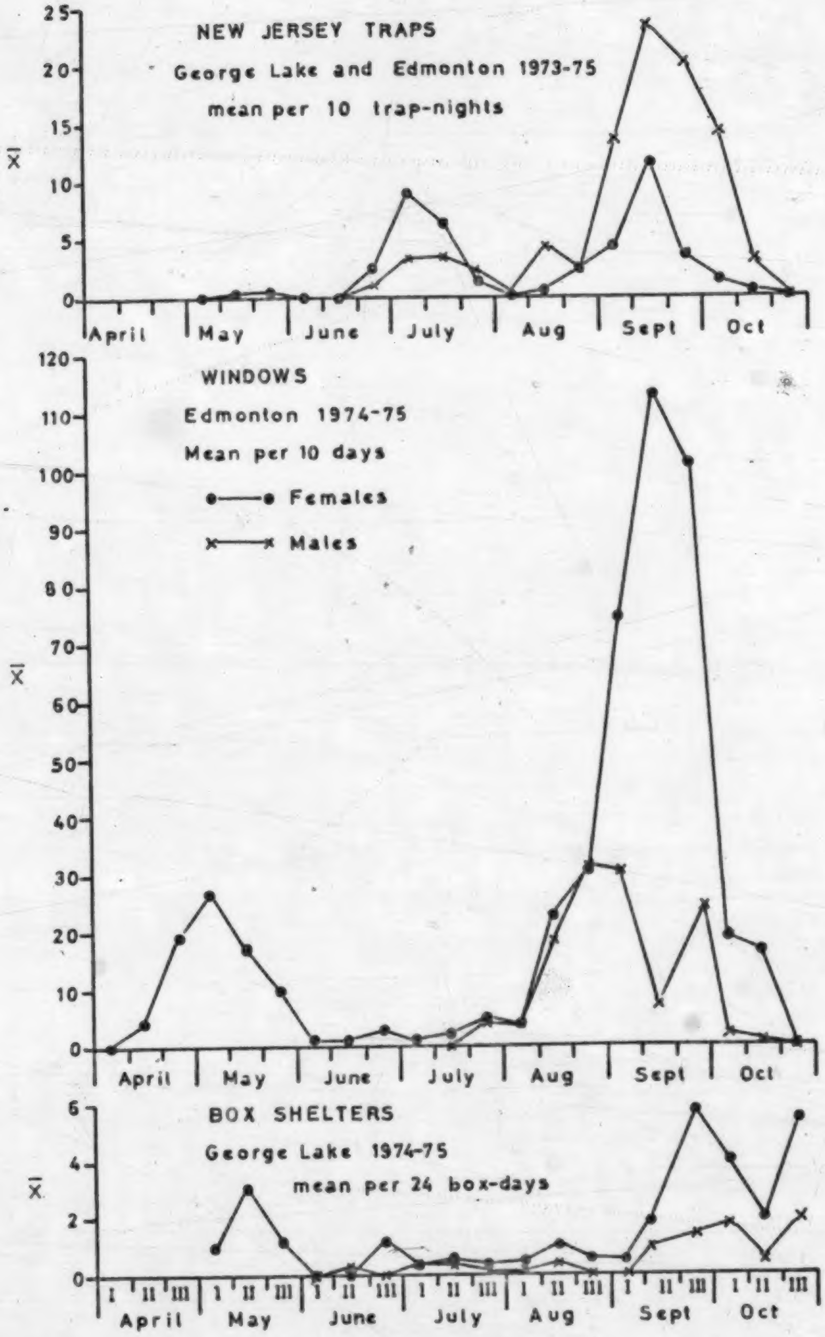


Fig. 16. Seasonal abundance of *Culex territans*.

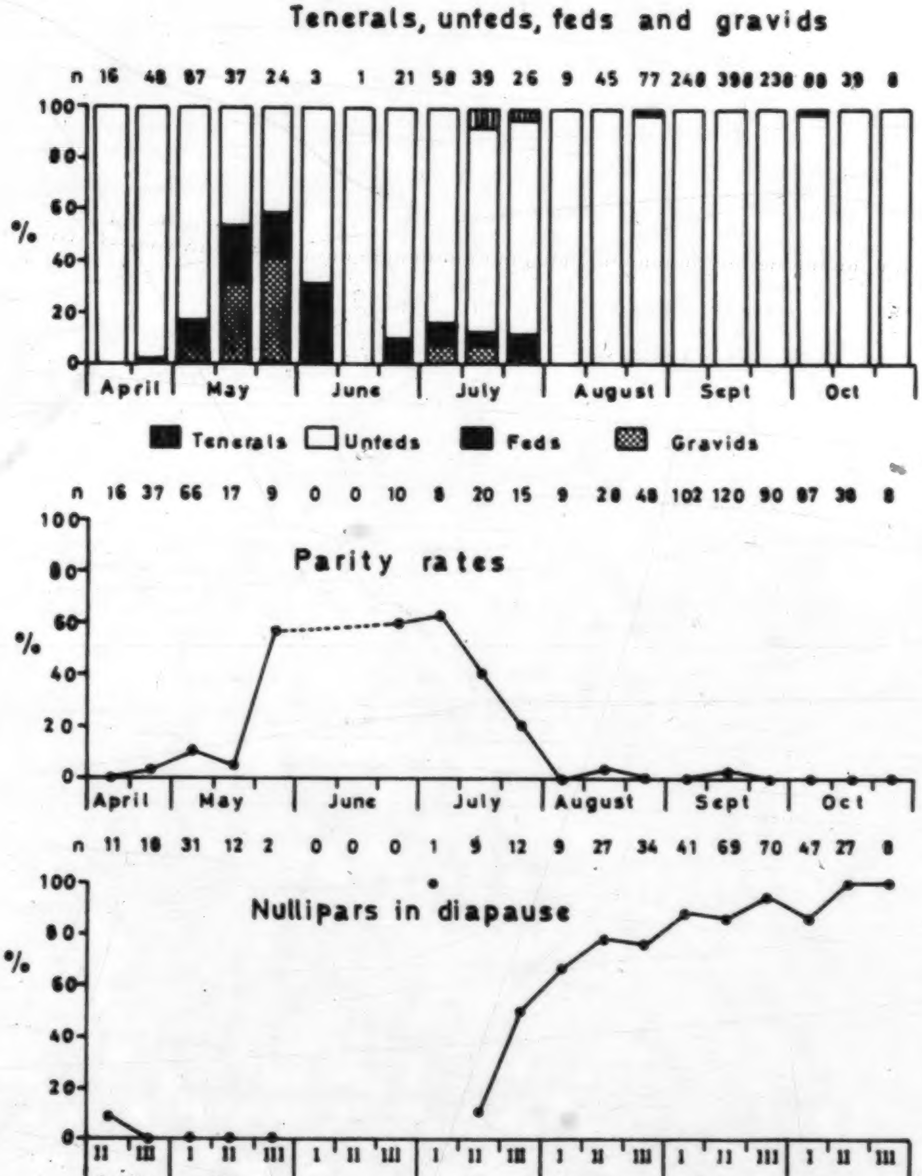


Fig. 17. Seasonal changes in fed, gravid, teneral, diapause and parous rates of *Clx. territans*.

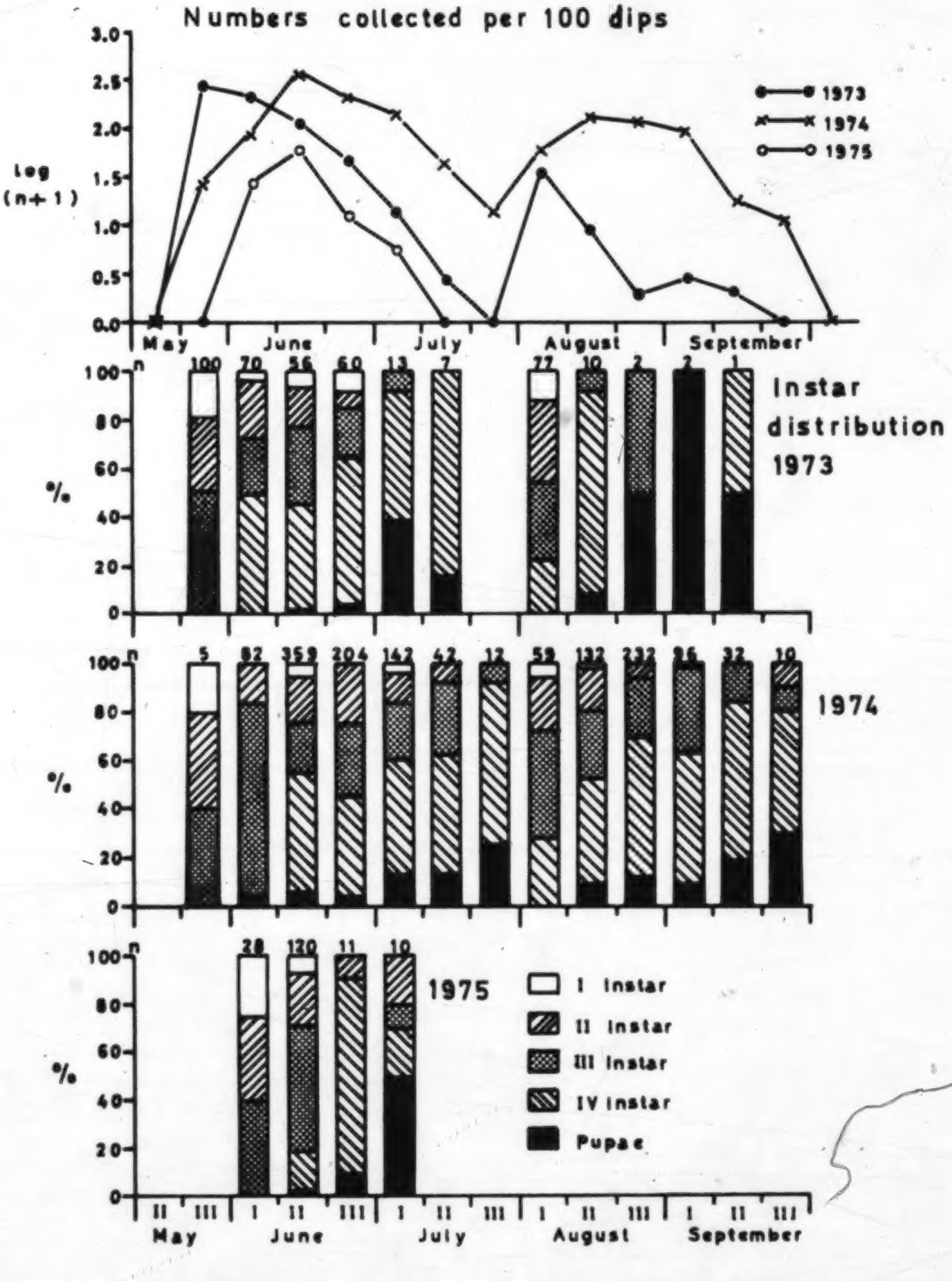


Fig. 18. Seasonal abundance and instar distribution of *Clx. territans* larvae and pupae in the lakeside pond.

July, came too soon for these to have been the offspring of the first summer generation and they were more likely the later part of it.

The first collections of larvae from the lakeside pond contained instars I, II and III, so they must have been there earlier, (Fig. 18), and larvae were found in mid-May in pool VIII (location in Fig. 2). Pupae were collected from mid-June to late July and from mid-August to late September. The distribution of pupae and the two peaks of abundance in 1973 and 1974 suggest two generations. In 1975 there was only one generation in the lakeside pond. Larvae were also found in pools I, II, IV and VIII.

The seven blood meals tested all gave negative results, but since all were fresh feds (Sella 2 or 3), it confirms that the meals were not frommammals or birds. Twenty-nine females had a mean of 91.8 eggs, (range 51 - 161, standard deviation 27.5). A par from a box shelter on 12/vii/74 had 49 mature eggs in one ovary and the other ovary in stage IIa. No retained eggs were seen in 37 other pars examined. Only 5 tenerals were dissected. None were inseminated, compared with 99% of 769 post-tenerals. Three of the 5 had follicles in stage N1, meconium present, and no fatbody development.

From April to June about half the nullipars had follicles in stage II, but from July to October almost all of them had follicles in stage I, (Table 13). About half the pars had follicles in stage II.

If we set an upper limit of 1.5 for the F:G ratios of diapausing females, 38% were in diapause in February and March, 7% in April and none in May, (Fig. 19). No females were measured in

Table 13. Seasonal changes in distribution of follicle stages of nulliparous and parous *Culex territans*.

	April to June		July to October	
	Nullipars	Pars	Nullipars	Pars
No. examined	122	16	555	16
% in stage N1	0.0	0.0	0.7	0.0
N2	0.0	0.0	2.2	0.0
I	42.6	56.2	96.0	37.5
IIa	44.3	43.8	0.7	50.0
IIb	13.1	0.0	0.4	12.5

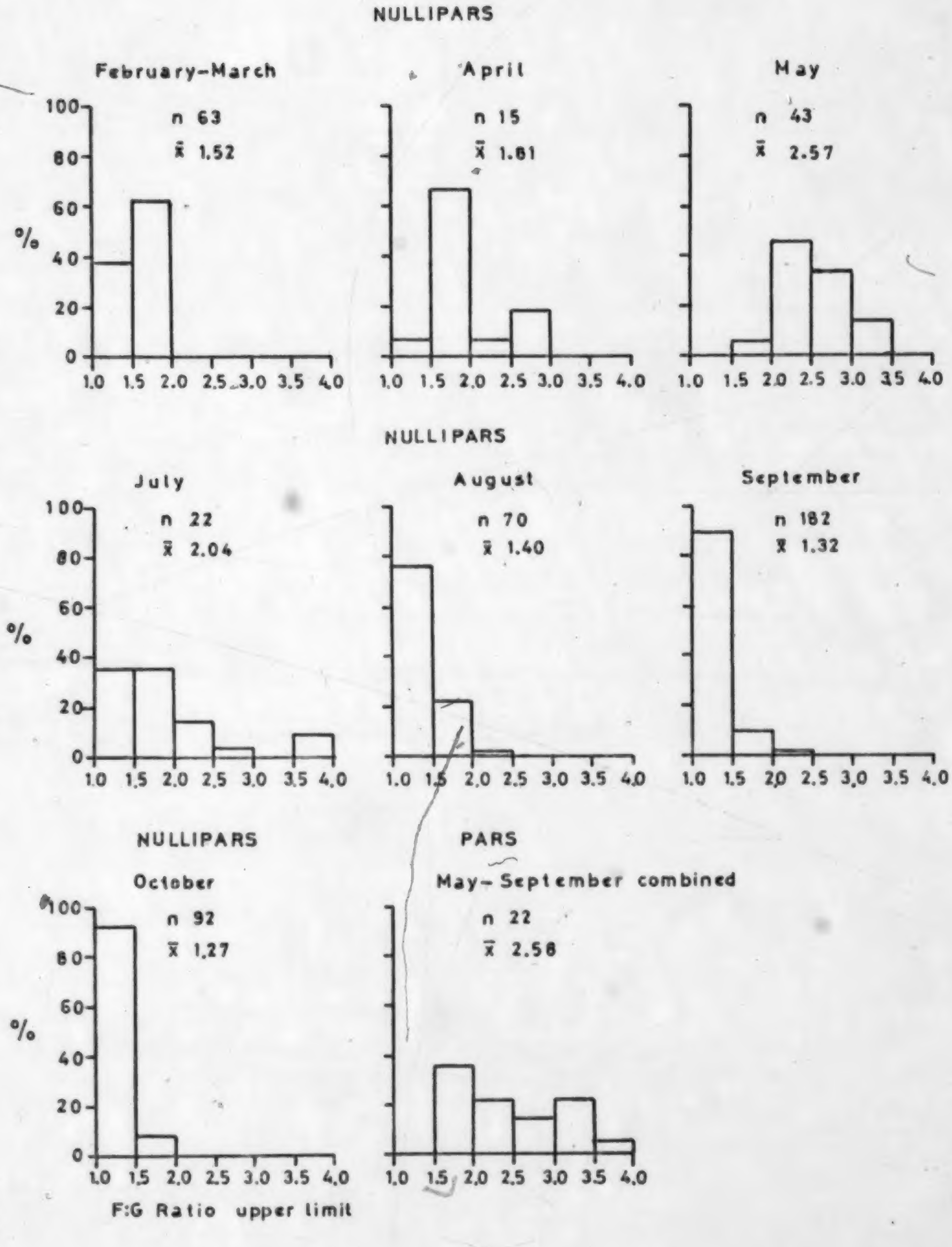


Fig. 19. Seasonal changes in distribution of F:G ratios of nulliparous *Clx. territans*. At sites other than bait.

June, but in July 36 % were in diapause, in August 76 %, in September 89 %, and in October 92 %. None of the pars was in diapause. This interpretation agrees well with the time of disappearance of feds and gravids, but makes 8 - 22 % of the unfeds from August to October "gonoactive". Raising the upper limit to 2.0 would include almost all of these in the diapausing group, but 36 % of the pars had F:G ratios of 1.6 - 2.0.

Most of the overwintered females still had well-developed fatbodies in May, and even pars in midsummer had enough fatbody to distend their abdomens, (Fig. 20). Fatbody accumulation in the population during August was slower than in *An. earlei*, but from late August onwards, most *Clx. territans* had abdomens distended with fatbody. Syrup was found in the crops of 3 % of the nullipars and 16 % of the pars, with the highest rates in June and July, (Table 14).

On 13/viii/74 a *Clx. territans* male was collected from a swarm of *Culiseta morsitans dyari* males, (see Section 3.13).

In the summary of seasonal development (Fig. 21) no blood feeding period is indicated since this activity was never observed, but from the collections of blood-feds it would be expected to last from late April to late July.

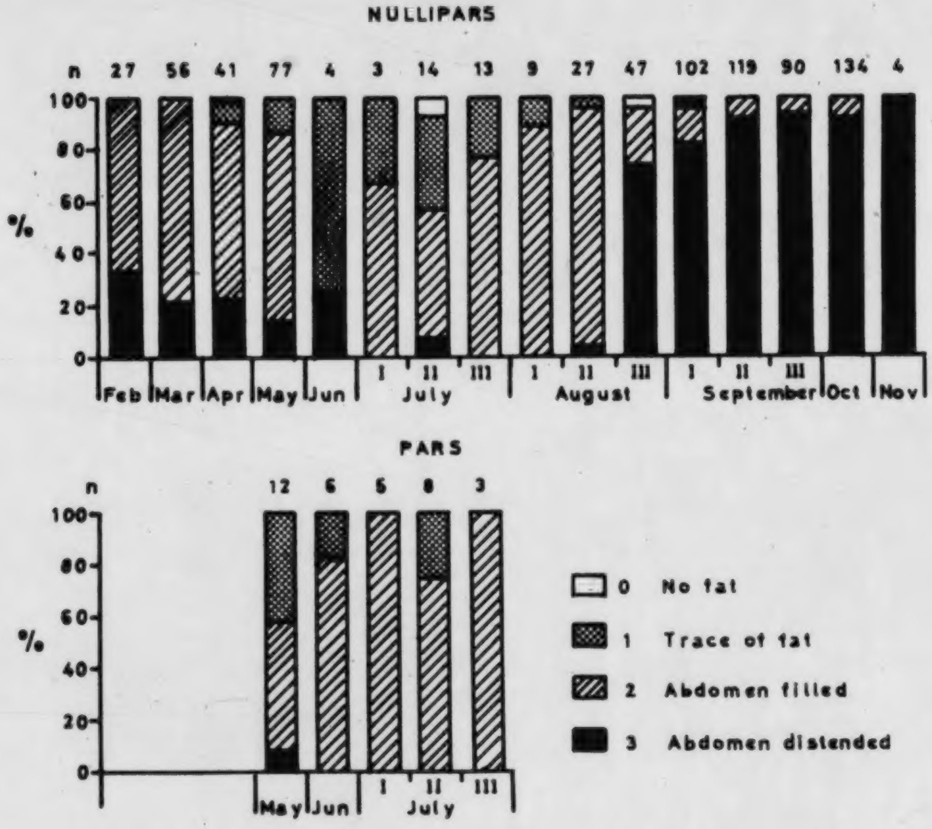


Fig. 20. Seasonal changes in fatbody ratings of nulliparous and parous *Clx. territans*.

Table 14. Seasonal changes in numbers of nulliparous and parous *Culex territans* with syrup in their crops.

Month	Nullipars			Pars		
	Number examined	with syrup No.	%	Number examined	with syrup No.	%
April	22	0	0	1	0	0
May	49	0	0	11	2	18
June	1	0	0	3	3	100
July	25	3	12	15	0	0
August	71	3	4	1	0	0
September	180	5	3	1	0	0
October	94	2	2	0	-	-
Total	442	13	3	32	5	16

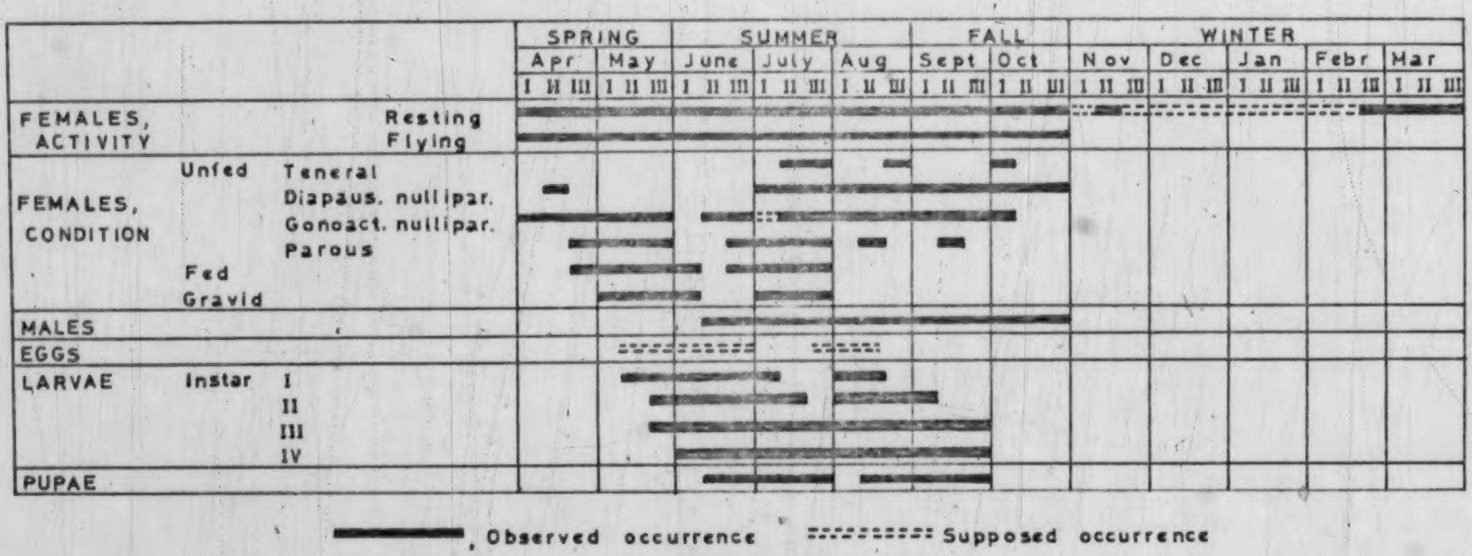


Fig. 21. Summary of seasonal biology of *Clx. territans*.

3.10. *Culiseta alaskaensis*

Females were seen on humans and windows from mid-April, on cattle from late April, and in the New Jersey traps and box shelters from mid-May, (Fig. 22). There was a spring peak of overwintered females in late May. With the emergence of the summer generation from late June, the numbers in the New Jersey traps and on windows rose to their main peak in mid-July, but no corresponding increase was seen in the numbers on cattle. There was a drop in catches in August, and a rise to a third small peak in late September and early October. There is no evidence to suggest that this was a second summer generation, and simply indicates a reduction in flight activity in late summer. The females may aestivate in central Alberta as they do in Alaska (Hopla, 1970). Males were first taken in mid-June, the highest numbers in mid-July and the last in mid-October. Even if these last males had been the offspring of the females taken at bait in late July, and they had taken a month in the aquatic stages (which were never found in August), they would still have been 6 - 7 weeks old when captured. Near Anchorage, Alaska Sommerman (1964) observed males swarming as late as 8/x.

The first feds were seen in late April and the first gravids and pars in early May, (Fig. 23). The decrease in the parous rate among the females at bait is an artifact of combining data for several years. In 1974 snowmelt was in mid-April and intense biting activity was observed less than 10 days afterwards. Snowmelt was also in mid-April in 1975 and around the end of March in 1976. In both years the first adults were not observed until 12 - 15 days after snowmelt,

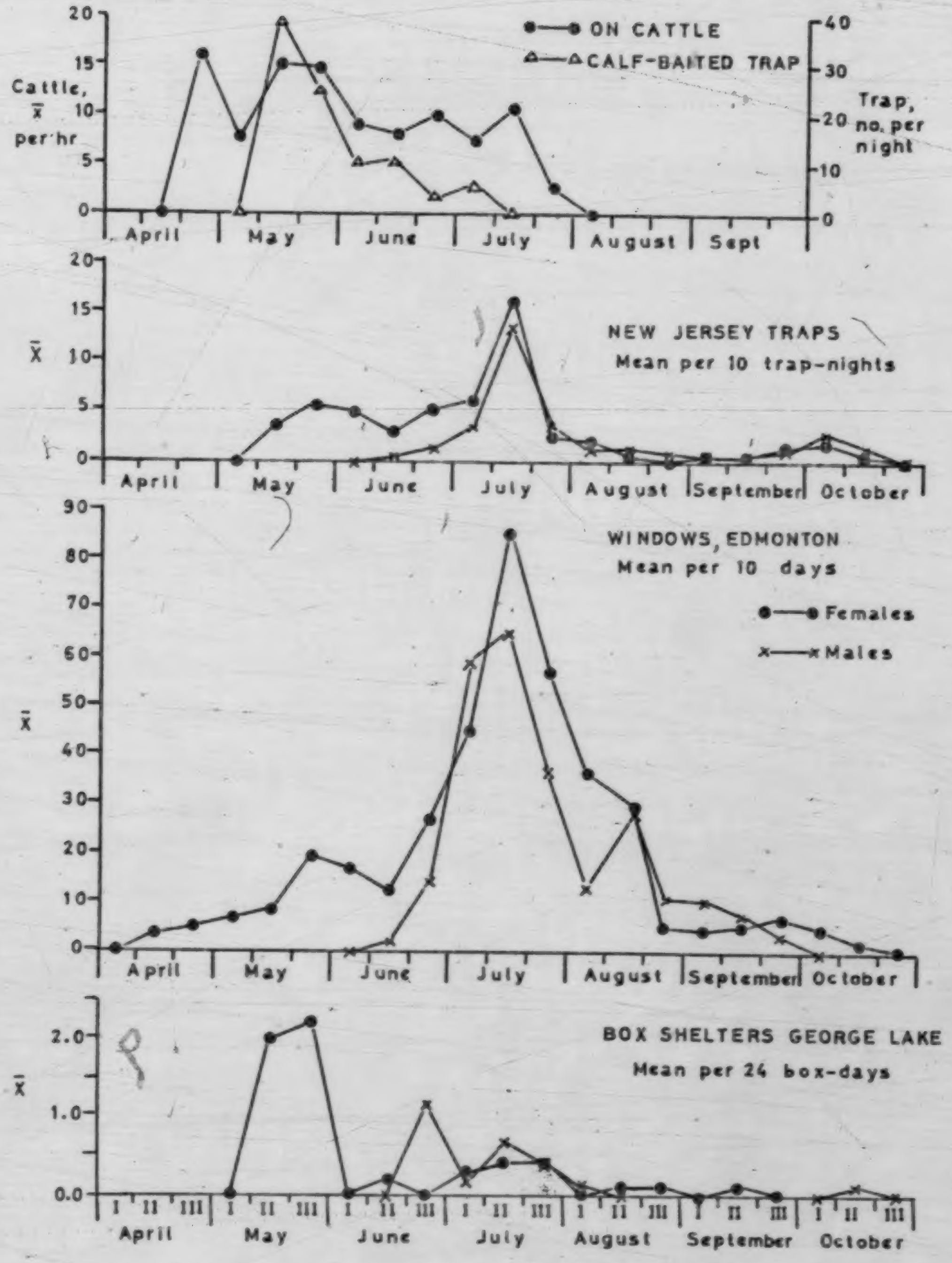


Fig. 22. Seasonal abundance of *Culiseta alaskaensis*.

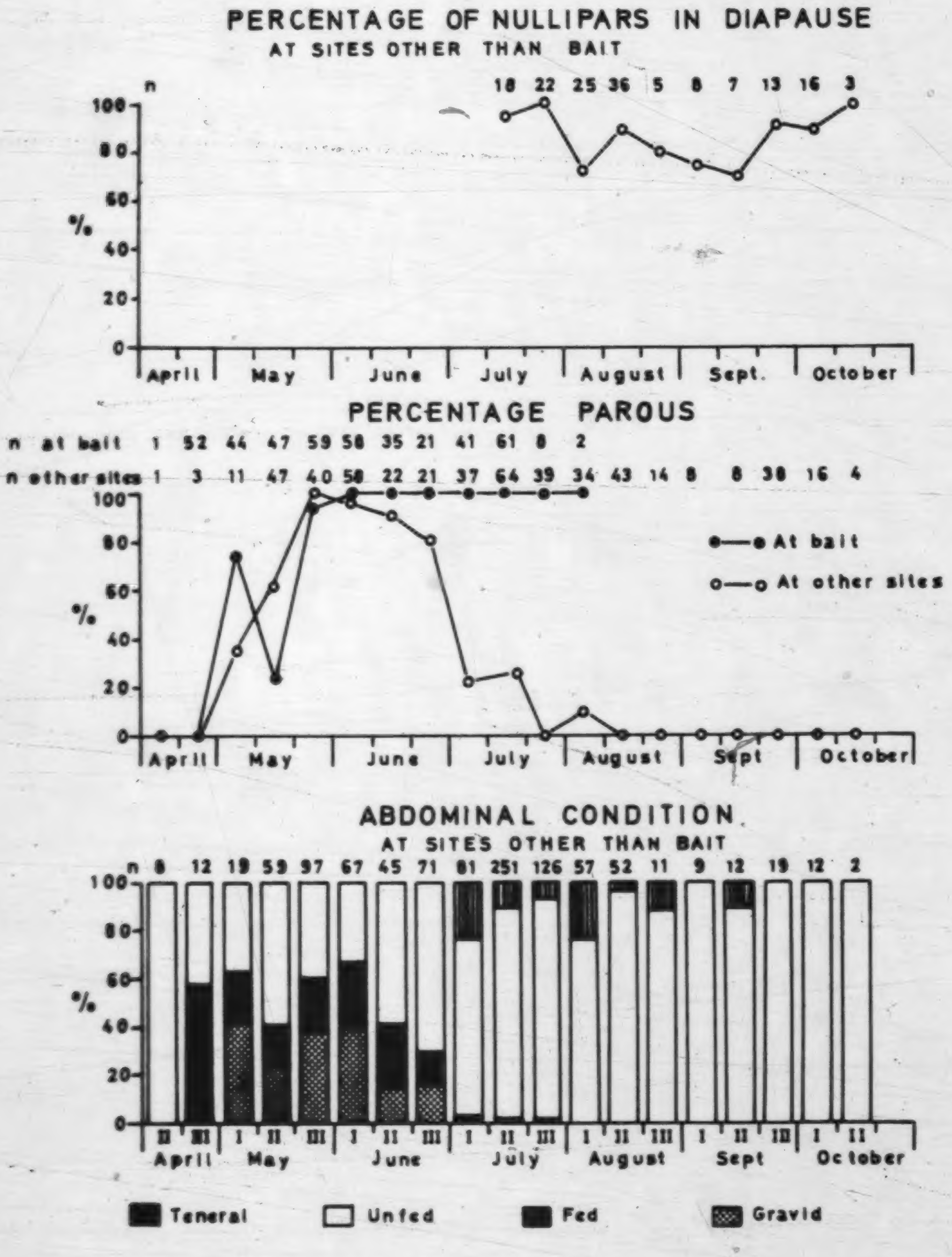


Fig. 23. Seasonal changes in fed, gravid, teneral, diapause and parous rates of *Cs. alaskaensis*.

(Fig. 24). In late May and early June most females collected at sites other than bait were fed, gravid or parous, (Fig. 23). Unfed nullipars appeared in June, but tenerals not until early July. Small numbers of feds and gravids were taken in July, presumably overwintered females since only pars were taken at bait from early June onwards. Only unfed, nulliparous females were taken from August through October. A teneral was taken as late as 11/ix/74, from a box shelter close to the lakeside pond.

The first larvae were taken in the lakeside pond in late May, the first pupae in mid-June and the last pupae in early August (Fig. 25). Larvae were found in pool IV on 14/vi/72, (location in Fig. 2), and also in a clear rock pool without vegetation, by a stream in a wooded ravine 4 km east of Devon, Alberta, on 6/vii/71.

The absence of nullipars at bait after the emergence of the summer generation and the absence of young larvae after the end of July both suggest that the summer generation do not take blood in the year they emerge, so that there is only one generation a year here, as in Alaska, (Frohne, 1954a).

From snowmelt up to mid-May, I noted several attacks on humans during the daylight hours, particularly while walking through the woods. During summer humans were rarely attacked unless working close to cattle. Females were seen attacking cattle as early as 10:00 hours on 24/iv/74, and a series of catches made in 1975 demonstrate the shift from biting both before and after sunset in May and June to biting only after sunset in July, (Fig. 26). The only *Cs. inornata*

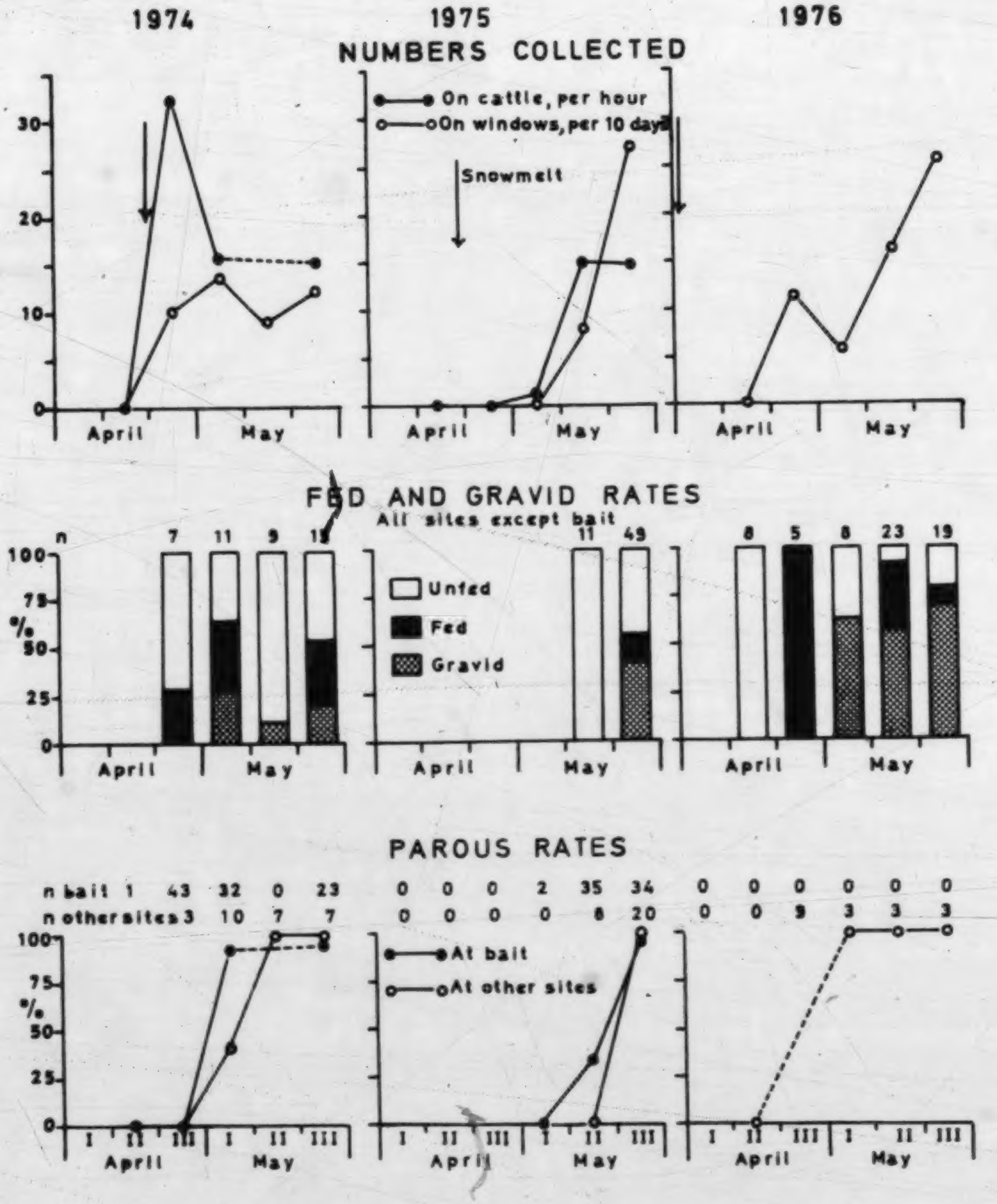


Fig. 24. Appearance of *Cs. alaskaensis* in spring, 1974, 1975 and 1976.

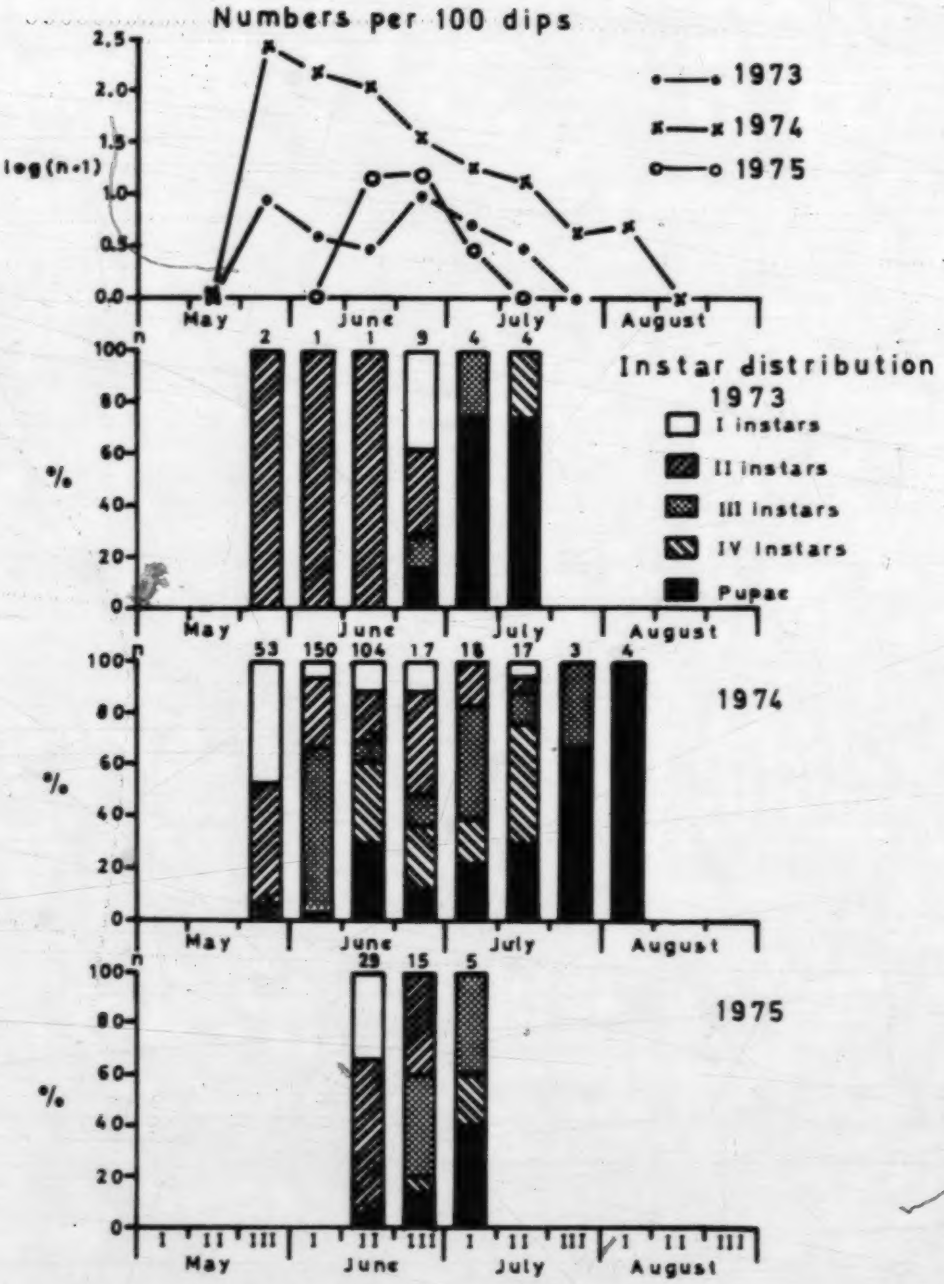


Fig. 25. Seasonal abundance and instar distribution of *Cs. alaskaensis* larvae and pupae in the lakeside pond.

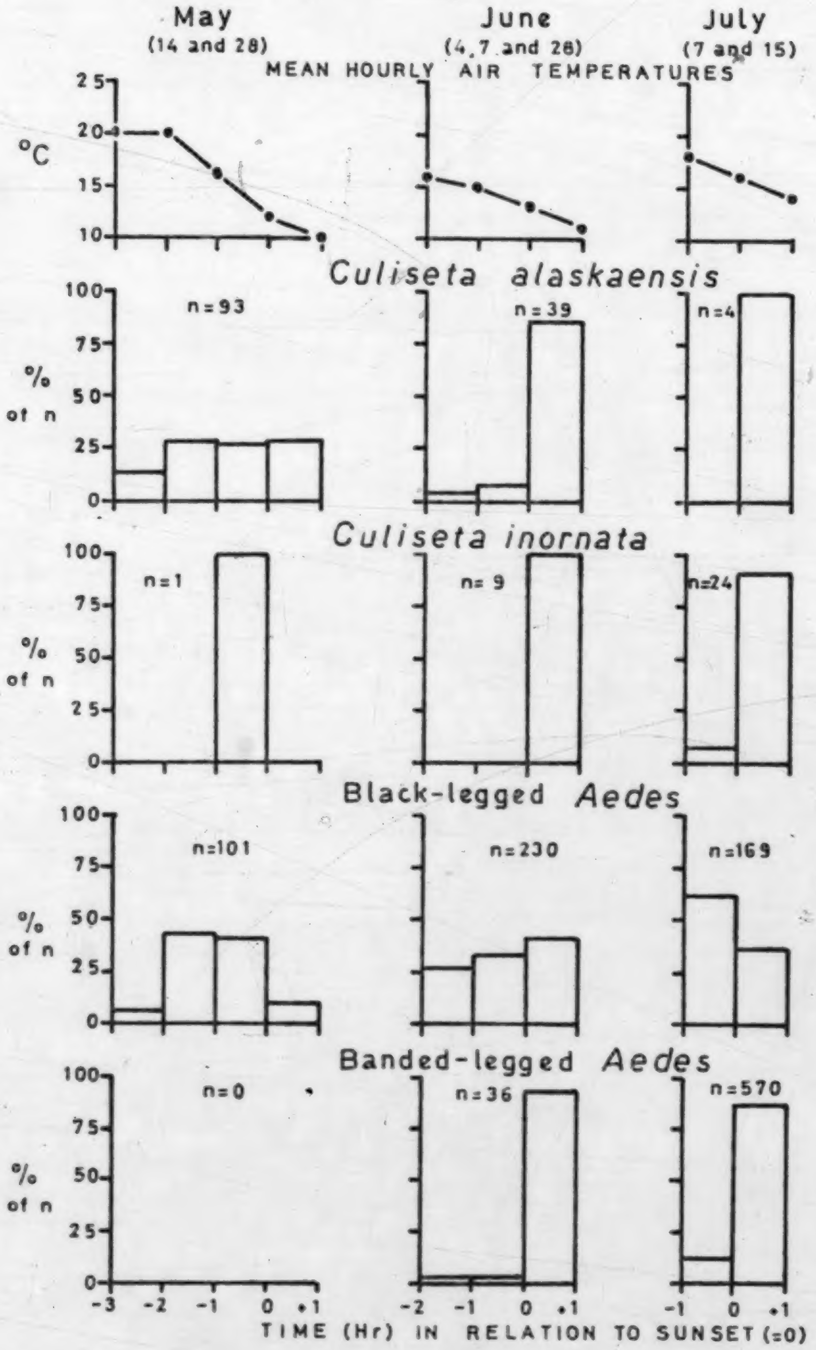


Fig. 26. Seasonal shift in numbers of *Culiseta* and *Aedes* females on cattle before and after sunset, 1975.

in May was taken in the hour before sunset, but later in the season they nearly all arrived after sunset. The black-legged *Aedes* attacked in numbers both before and after sunset, and the banded-legged *Aedes* almost all attacked after sunset. Frohne (1954a) states that in Alaska *Cs. alaskaensis* bites earlier in the spring. At Nagasaki, Japan, Mogi et al (1970) noted a similar shift in the peak time of collections of *Culex tritaeniorhynchus* at dry ice, from sunset in spring to after sunset in summer.

All 9 blood-feds from George Lake, (3 from the New Jersey trap and 6 from the box shelters) were positive for bovines. Of the 11 blood-feds from the windows at Edmonton, 9 were positive for bovine, 1 for rabbit and 1 negative. Females were observed attacking pigs and lambs at George Lake on the evening of 22/v/74. In Alaska, Hopla (1965, 1970) observed females feeding on dogs, cows, horses, snowshoe hares and bears, and they seemed to prefer hares and rabbits to rats, voles or birds as bait in traps. In the calf-baited trap at George Lake, 81 of 94 (86 %) were engorged, but neither of the two females in the quail-baited trap were engorged.

Sixty-three females had a mean of 183.4 eggs (range 103 - 313, standard deviation 46.7). The mean number of eggs was 210 in May (32 females), 158 in June (30 females), and the female collected on 22/vii had 154 eggs in what must have been at least her third gonotrophic cycle. Out of 484 pars examined, 80 had retained eggs (16.5 %), 50 with 1, 13 with 2, 7 with 3, 2 with 4, 6 with 5 - 10, 1 with 11 - 15 and 1 with 16 - 20; the mean number was 2.27. In two studies in Alaska with wild-caught females fed on humans, the mean numbers of eggs produced were 183 (Frohne, 1954a) and 138.5 (Sommerman, 1969). The smaller number may have been due to suboptimal feeding conditions, since some blood was passed undigested, and half the females took more than one meal before producing eggs.

Two unfed nulliparous females collected from the windows on 29/vii/75 and 12/viii/75 had follicles as large as normal stage III follicles (170 μm long), but filled with a yellowish-brown material unlike yolk. One female (29/vii) had 8 such follicles in one ovary, 13 in the other, and the remainder in stage I. In the other female most of the follicles seemed to be affected.

Of 1030 females dissected, 4 (0.39 %) had golden, thick-walled oval bodies in their ovaries, resembling resting sporangia of *Coelomomyces* (Phycomycetes, Blastocladiales) as figured by Steinhaus, (1949, Fig. 104L and 105D). The ovaries were swollen and yellow. Three sporangia had mean dimensions of 55 x 34 μm. Two of the infected females were taken at cattle on 22/v/74 and one on 28/v/75, and the fourth on a window on 29/vi/75. The infected females were well enough to fly to the sites they were collected and 3 of them were at bait, but Jenkins (1964) states that egg development is suppressed in infected females. Infected larvae usually die before pupation, and infections in adults are usually confined to the ovaries, (Couch and Umphlett, 1963). *Coelomomyces psorophorae* is found in *Culiseta inornata* larvae in southern Alberta, (Shemanchuk, 1959b), but Jenkins (1964) gives no records of infections in *Cs. alaskaensis* anywhere.

The alternate host for *C. psorophorae* is *Cyclops vernalis* (Whisler, Zebold and Shemanchuk, 1975).

There were 29 tenerals among 330 females collected from the windows (8.8 %). Only 12 % were inseminated, 95 % had follicles in stage N and 49 % had meconium. There was some fatbody development in 36 % and 46 % had syrup in their crops. The nearest known breeding site was a permanent woodland pool by the river bank, about 1 km from the windows, where a IV instar larva was found on 3/vi/72. Thus in *Cs. alaskaensis* the tenerals do not only rest near the breeding sites but may fly quite far, and take nectar. *Aedes taeniorhynchus* in Florida migrate when 6 - 24 hours old; some may still be teneral. Migration is sometimes preceded by flower-feeding and copulation, though the females are not inseminated, (Provost, 1974).

Most nullipars at bait had follicles in stage II and F:G ratios never less than 2.1, mostly 2.6 or more, (Fig. 27). Most pars, at bait and at other sites, had follicles in stage I and about one third of them had F:G ratios of 1.6 - 2.0. The nullipars at sites other than bait showed the biggest range of follicle stages and F:G ratios. All overwintered nullipars collected in mid-May had F:G ratios of 1.6 or more and 87 % had F:G ratios of 2.1 or more, (Fig. 28). Most females of the summer generation collected in July had F:G ratios of 1.5 or less, but from August through October most were in the range 1.6 - 2.0, and a few in the range 2.6 - 3.0. No sign of blood feeding was seen in nature during these months, and the few females that fed in the laboratory did not produce eggs. Thus the upper limit

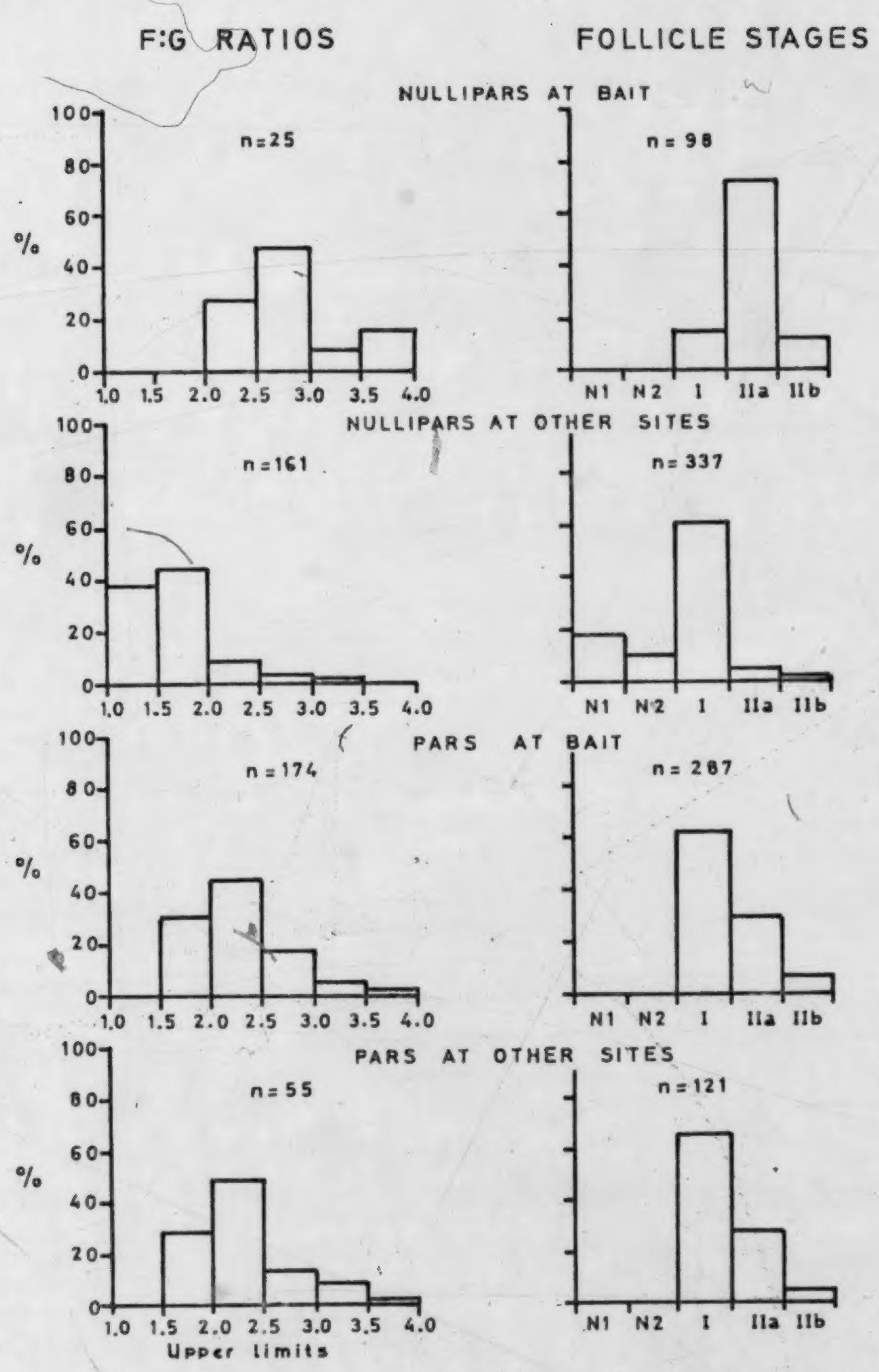


Fig. 27. Distribution of F:G ratios and follicle stages of *Cs. alaskaensis* in relation to parity and biting activity.

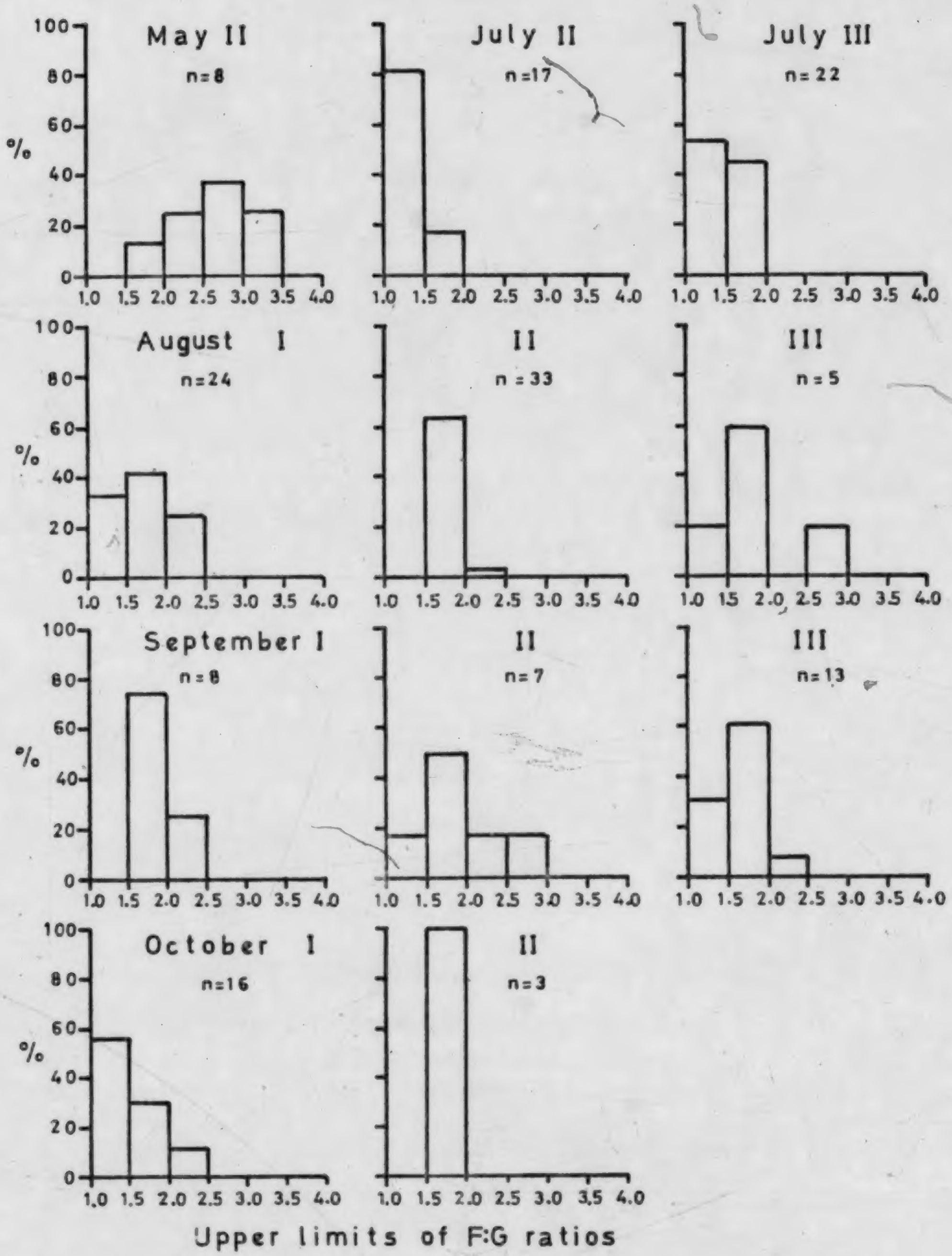


Fig. 28. Seasonal changes in distribution of F:G ratios of nulliparous *Cs. alaskaensis*. At sites other than bait.

for females in diapause should certainly be as high as 2.0 and possibly higher. Four of 100 nullipars in August, 1 of 55 (2 %) in September, and 3 of 20 (15 %) in October had follicles in stage II, indistinguishable from the follicles of females caught at bait in spring.

In April and May the nullipars still had well-developed fatbodies, but in the pars the fatbodies were depleted, (Fig. 29). Most nullipars collected from July to October had well-developed fatbodies, rated 2 or 3.

Syrup was found in nullipars from May to October and in pars from May to July, with the highest rates in June, (Table 15). Two out of 6 females collected from the windows and New Jersey traps from 3 - 6/v/74 had syrup in their crops. *Petasites* (coltsfoot) and *Taraxacum* (dandelion) flowered as early as this, but females were never seen on either plant. The syrup may have been the remains of large meals taken the previous year. In a few females the crop was so full that it distended the abdomen, (1 par in May and 2 in June, 3 nullipars in July and 4 in August). A female collected from a window on 31/viii/74 and dissected after 2 days storage at +2 C had several orthorhombic water-soluble crystals in her crop. This crystallization may have occurred during storage at +2 C. The absolute minimum air temperatures at George Lake in early September were +2 C or below in all 3 years 1973 - 75.

Noteworthy features of the seasonal development of *Cs. alaskaensis* (Fig. 30) are the single generation, the late date at which the last pars were taken biting, (they were probably a year old

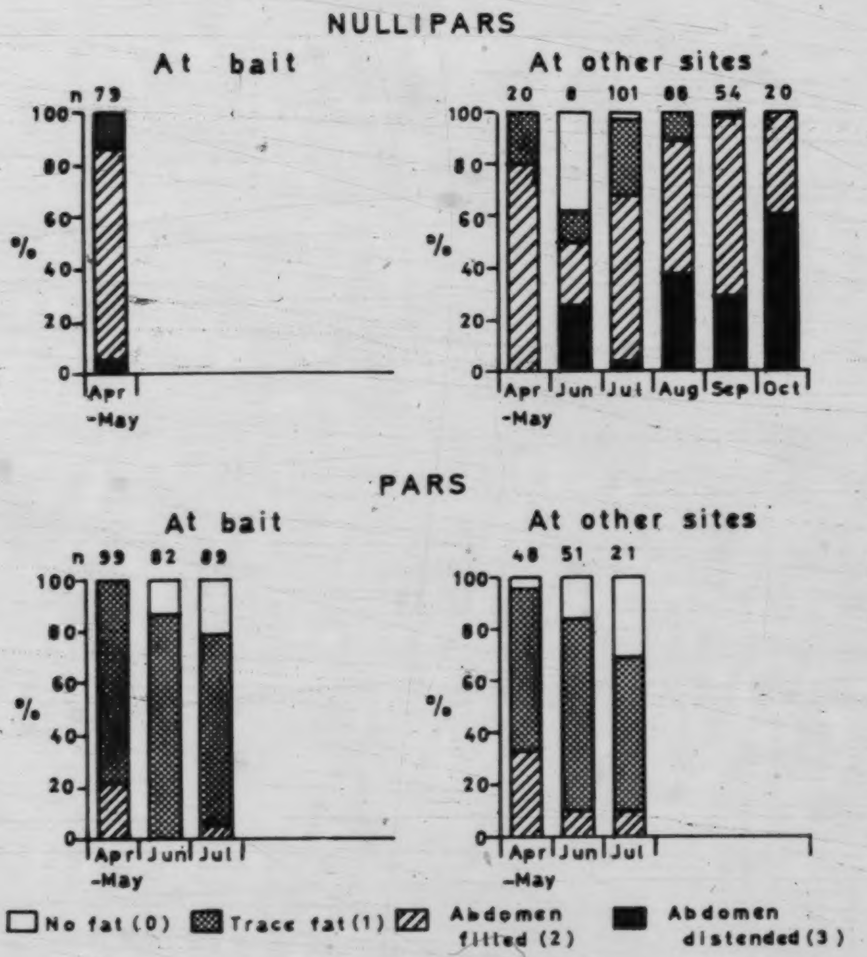


Fig. 29. Seasonal changes in fatbody ratings of *Cs. alaskaensis* in relation to parity and biting activity.

Table 15. Seasonal changes in numbers of nulliparous and parous *Culiseta alaskaensis* with syrup in their crops.

Month	Nullipars			Pars		
	No. exam.	with syrup No.	with syrup %	No. exam.	with syrup No.	with syrup %
April	42	0	0	0	-	-
May	41	13	32	130	23	18
June	6	4	67	114	48	42
July	71	23	32	102	35	35
August	69	17	25	3	0	0
September	31	8	26	0	-	-
October	19	10	53	0	-	-
Total	279	75	27	349	106	30

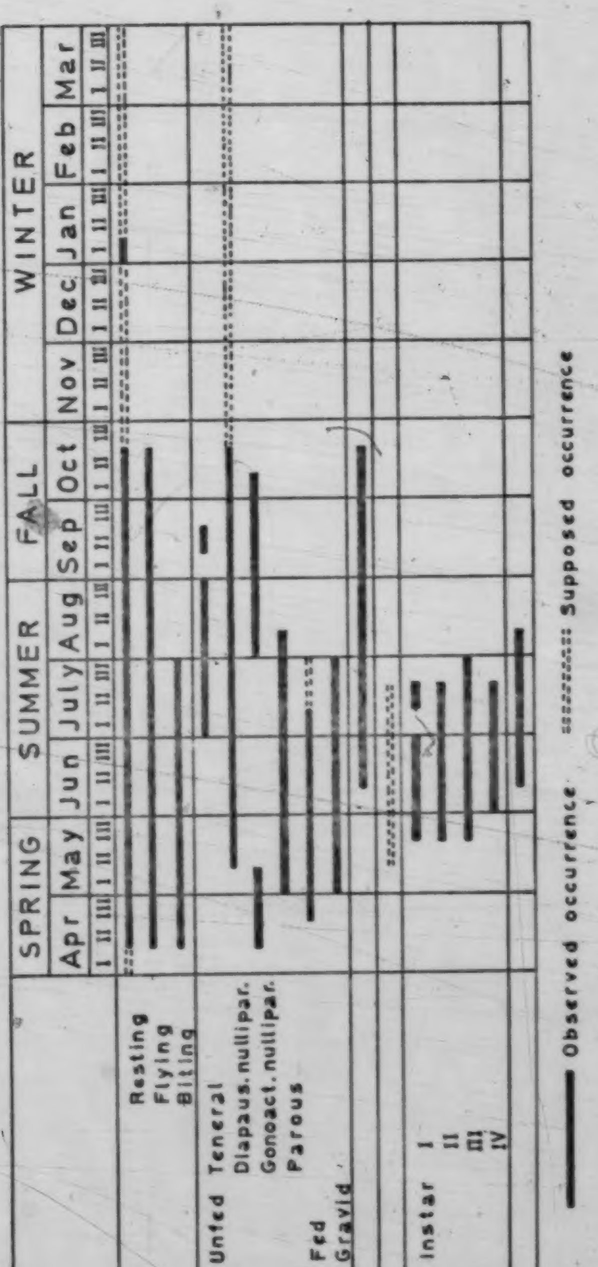


Fig. 30. Summary of seasonal biology of *Cs. alaskaensis*.

by this time), and the collection of apparently non-diapausing nullipars from August to October, though no blood feeding was detected during this period.

3.11. *Culiseta impatiens*

I only recognised two specimens, both males, one from a box shelter at George Lake on 26/ix/74 and the other from the New Jersey trap at Edmonton on 6/x/75. Some *Cs. impatiens* females may have been sorted to *Cs. inornata*; Frohne (1953) states that it is frequently impossible to distinguish the females. The two males were placed with *Cs. morsitans dyari* until their terminalia were examined. *Cs. impatiens* and *Cs. alaskaensis* have the same life cycle in Alaska, (Frohne, 1954b), and it is likely that they do in central Alberta also. My data for *Cs. inornata* do not indicate any biting activity at the time of snowmelt, nor emergence of diapausing females in June and July, thus it is unlikely that many *Cs. impatiens* were included with them.

3.12. *Culiseta inornata*

3.12.1. Seasonal abundance of adults

The earliest females at bait cattle were taken in late May, (Fig. 31), about one month after the first *Cs. alaskaensis*, and in small numbers. None were taken in mid-June, but there was a sudden increase in late June, and many until mid-August. The highest attack rate ever observed was 216 females on a single calf during the first 30 minutes after sunset on 6/viii/74. There were far fewer in late August, and none were taken in September, though *Aedes spp*. were taken until mid-September. The last days that any were taken at bait were 23/viii/73, 21/viii/74, and 26/viii/75.

The first females in the New Jersey traps were taken in mid-May (earliest date 13/v/73) and on the windows in late May (earliest date 30/v/75). The earliest female seen anywhere was under a log at George Lake on 9/v/73. Numbers of females at sites other than bait were small until late June, then increased to a peak in late July at George Lake and in early August at Edmonton. The capture of males from mid-June onwards suggests that this increase in females represented the emergence of the first summer generation. The numbers of females declined in August and then rose to a second peak in September, which suggest a second summer generation. The light trap data indicate an increase in nocturnal flight activity after the last females were taken at bait. A few males and females were taken in the New Jersey traps in late October, the latest female on 30/x/73 and the latest male on 26/x/74.

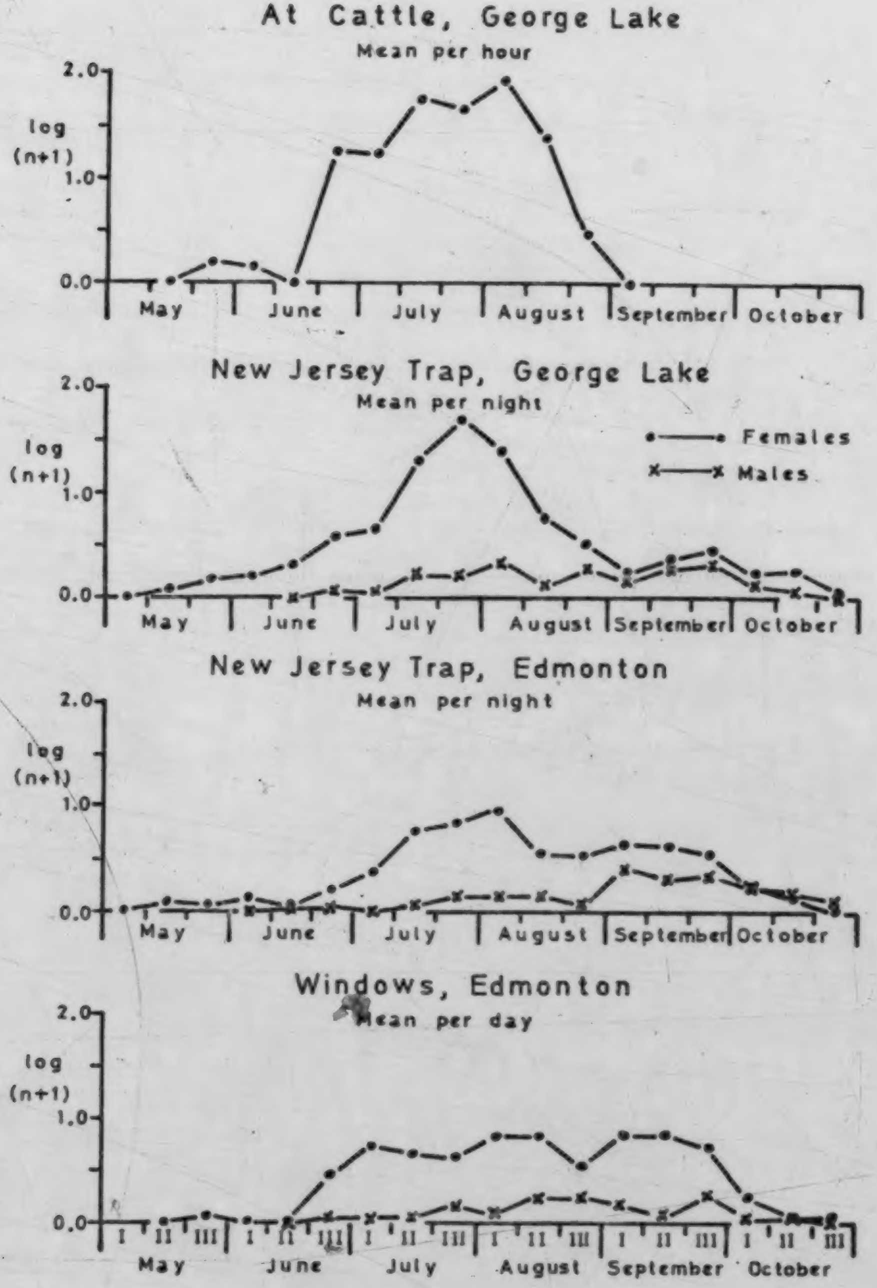


Fig. 31. Seasonal abundance of *Culiseta inornata*, 1973-75.

Relative abundance in 1973, 1974 and 1975 is shown in Figures 32, 33 and 34 respectively. Each year the New Jersey trap at Edmonton caught fewer than the trap at George Lake in summer but more in fall. The numbers in the New Jersey traps were consistently higher in 1973 than in the other years and the largest single catch was 697 females on the night of 14 - 15/vii/73, one night before full moon. In 1975 more females were usually taken from the calf outside, in the first hour after sunset, (Fig. 34), than were taken in the calf-baited trap, used all night (see also section 3.1.2.).

The New Jersey traps never caught mosquitoes before mid-May, though *An. earlei, Cs. alaskaensis* and *Clx. territans* flew before this. The surprising thing about *Cs. inornata* is that none were found by any method until about 7 weeks after snowmelt, (5 weeks in 1972, 7 in 1973, 9 in 1974, 6 in 1975 and 8 in 1976).

3.12.2. Seasonal changes in composition of catches

The female taken in early May was an unfed nullipar. Of the others taken in May and June all those at bait were parous and almost all those at other sites were unfed pars, feds and gravids, (Fig. 35). The first nullipars of the summer generation appeared in early July in 1973 and 1975 but not until late July in 1974, (Table 16). The first tenerals were not taken until late July. As the numbers at bait decreased during August the proportion parous increased which suggests that fewer nullipars were being recruited to the biting population. Very few pars or gravids were taken after August, the last pars in early September and the last gravids in

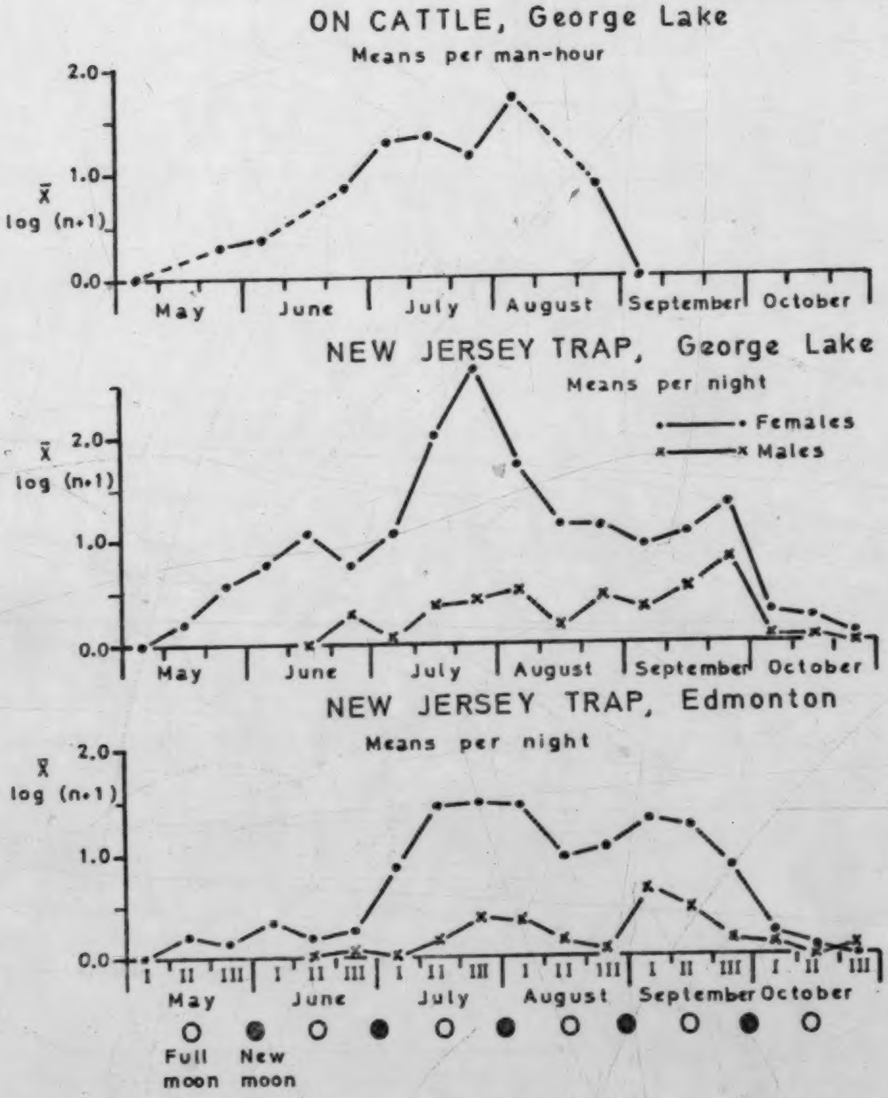


Fig. 32. Seasonal abundance of *Cs. inornata*, 1973.

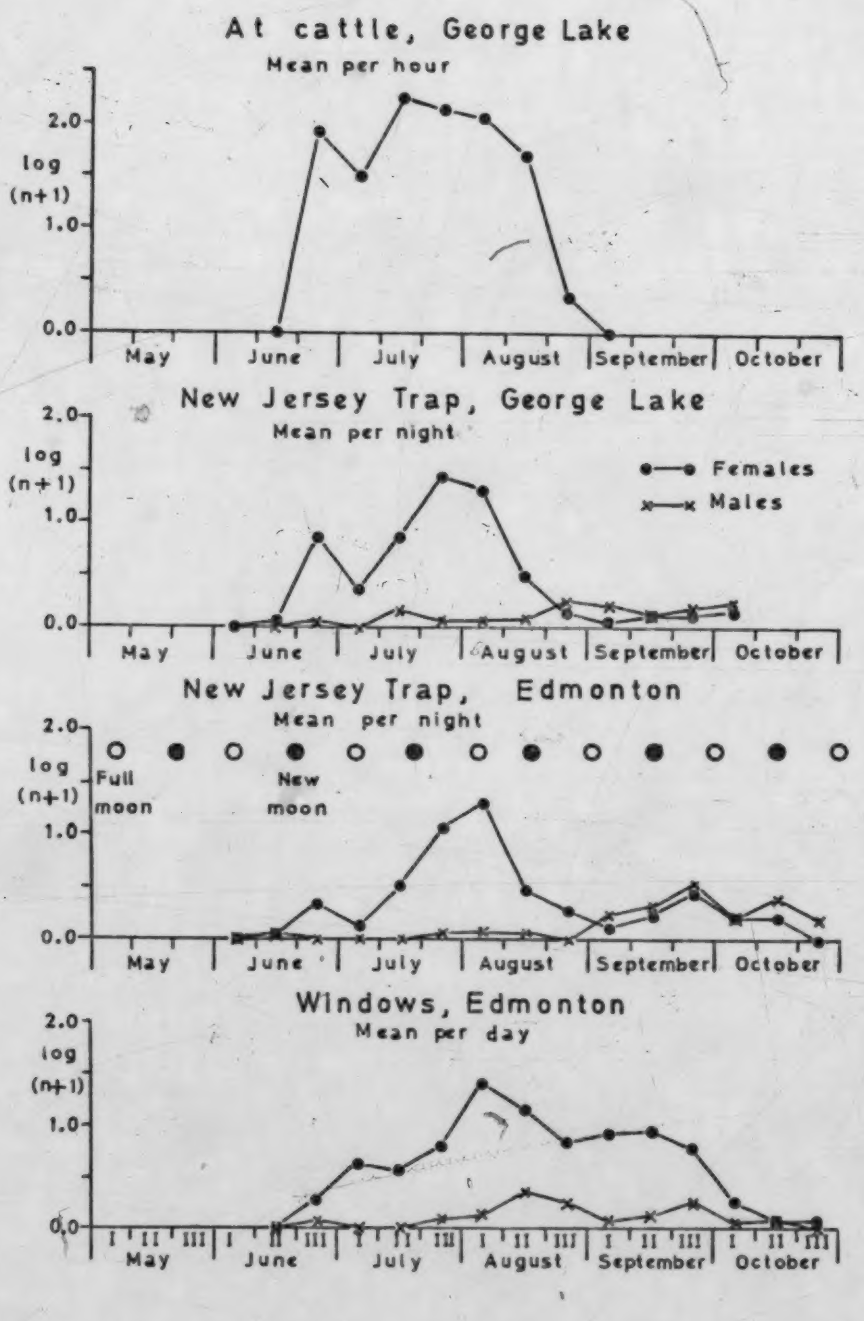
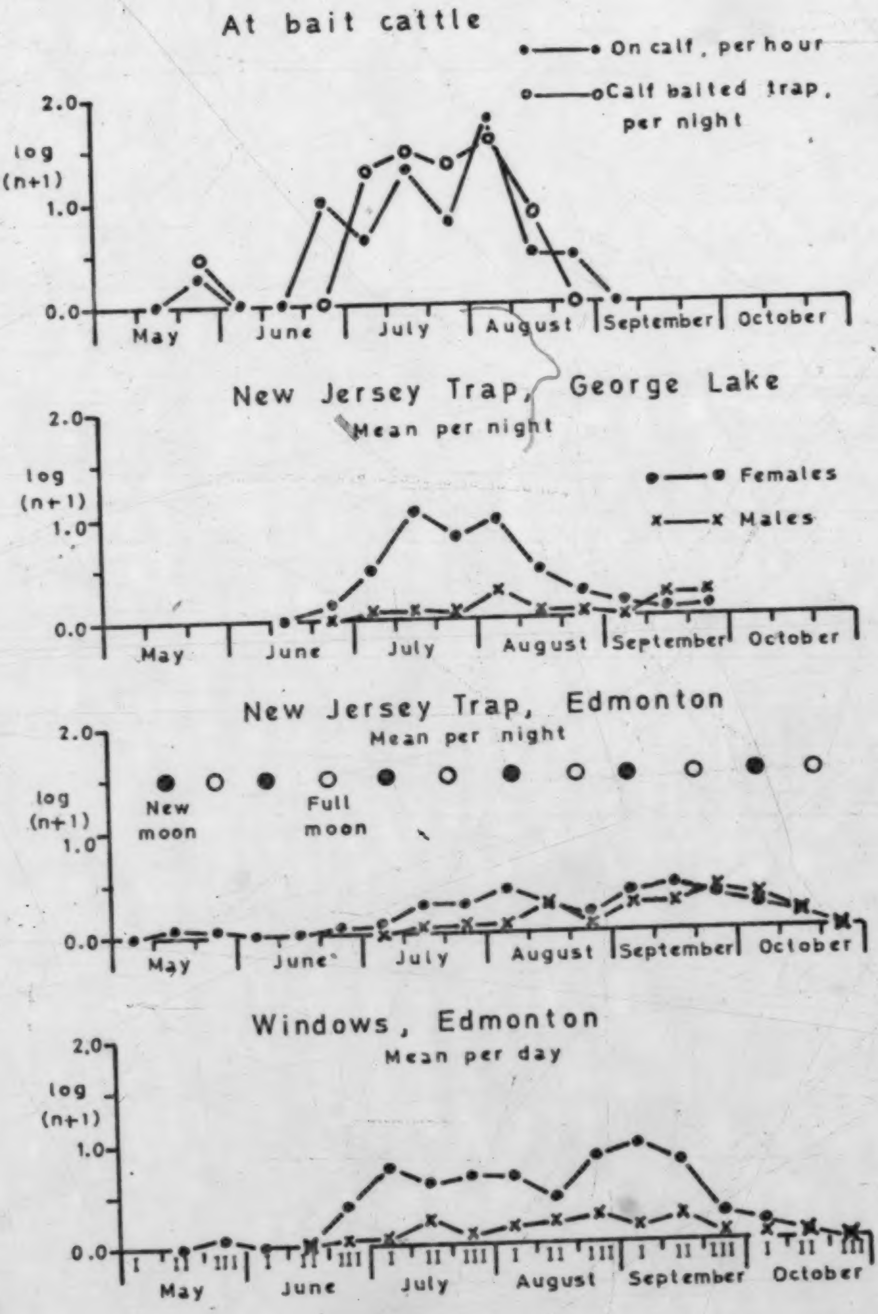


Fig. 33. Seasonal abundance of *Cs. inornata*, 1974.



At bait cattle

New Jersey Trap, George Lake
Mean per night

New Jersey Trap, Edmonton
Mean per night

Windows, Edmonton
Mean per day

Fig. 34. Seasonal abundance of *Cs. inornata*, 1975.

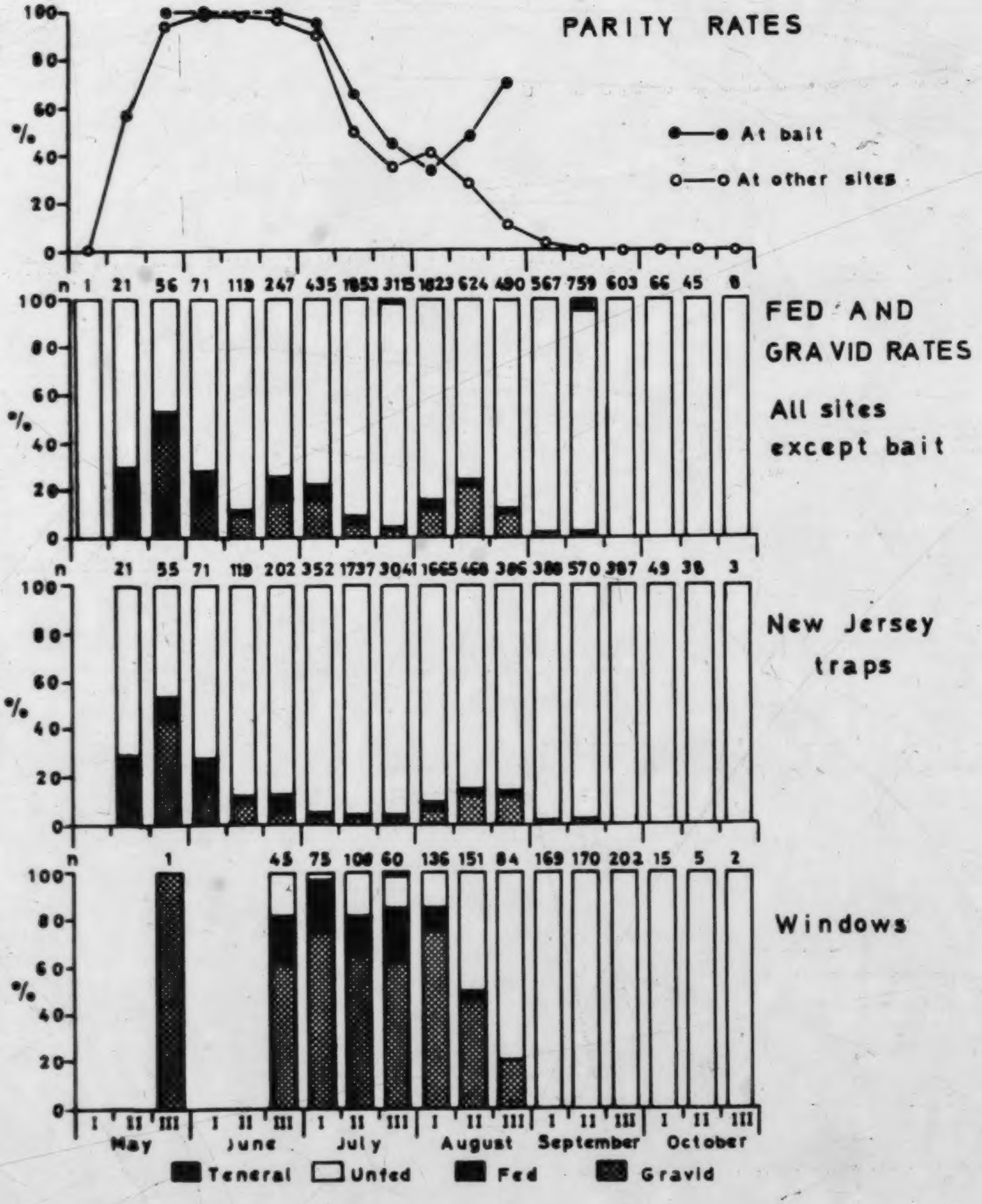


Fig. 35. Seasonal changes in fed, gravid, teneral and parous rates of *Cs. inornata*.

Table 16. Parity rates of *Culiseta inornata* at bait and at other sites, 1972-75.

	1973			1974			1975			All years (incl.1972)		
	Diss. No.	Par No.	%	Diss. No.	Par No.	%	Diss. No.	Par No.	%	Diss. No.	Par No.	%
At bait												
May III	0	-	-	0	-	-	3	3	100	3	3	100
June I	2	2	100	0	-	-	0	-	-	2	2	100
III	15	15	100	35	35	100	9	9	100	59	59	100
July I	21	10	83	26	26	100	24	23	96	62	59	92
II	41	16	39	26	26	100	14	12	86	81	54	67
III	7	3	43	88	28	32	26	24	92	121	55	46
Aug I	17	3	18	50	21	42	17	4	24	84	28	33
II	0	-	-	59	31	52	9	2	22	68	33	48
III	23	20	87	15	7	47	1	1	100	39	28	72
At other sites												
May I	1	0	0	0	-	-	0	-	-	1	0	0
II	14	8	57	0	-	-	0	-	-	14	8	57
III	27	25	93	0	-	-	0	-	-	27	25	93
June I	51	50	98	0	-	-	0	-	-	51	50	98
II	84	83	99	1	1	100	0	-	-	85	84	99
III	79	75	95	36	36	100	17	17	100	132	128	97
July I	156	138	88	18	17	94	27	27	100	201	182	90
II	131	32	24	40	34	85	48	41	85	219	107	49
III	146	70	48	85	10	12	62	22	36	293	102	35
Aug I	132	45	34	126	58	46	52	25	48	310	128	41
II	121	37	31	70	30	43	54	2	4	245	69	28
III	157	23	15	53	4	8	48	4	8	299	32	11
Sept I	135	3	2	60	0	0	48	0	0	308	3	1
II	135	0	0	40	0	0	34	0	0	239	0	0
III	136	0	0	59	0	0	31	0	0	240	0	0
Oct I	36	0	0	21	0	0	16	0	0	86	0	0
II	89	0	0	10	0	0	6	0	0	105	0	0
III	4	0	0	4	0	0	0	0	0	8	0	0

mid-September. The New Jersey traps caught few feds or gravids, except in late May, so their disappearance in fall was not so striking as it was in the window collections, where they predominated during summer.

3.12.3. Larvae and pupae

The first larvae were collected in the lakeside pond in early June and the first pupae in late June, (Fig. 36). The first summer generation was about a decade behind *Cs. alaskaensis* at the same site. There was a distinct break between the generations in late July, and first instar larvae of the second summer generation appeared in August. Larvae were most abundant in late August 1973 and mid-August 1974. In 1973 a few pupae were collected in October, the last on 31/x, one day before permanent frost. A search was made on 6/xi through a hole in the ice but none were found. In 1974 the last larvae and pupae were taken in mid-September, and none were found in 1975, though the pond was searched from May to September. Larvae were also in the Lakeside pond in great abundance in August and September 1972. On 13/vii/73 they were also found in two sloughs in pasture, (III and VI in Fig. 2).

Five larvae collected from the lakeside pond on 30/viii/72, 16/viii/73, 30/viii/73 (two), and 29/ix/73, appeared puffy and sluggish and failed to pupate after a long time in the laboratory. Three were examined and diagnosed as infected with *Thelohania inimica* (Protoza: Microsporida), by Dr. E. I. Hazard, U.S.D.A., Gainesville, Florida. This species was first described by Kellen and Wills (1962)

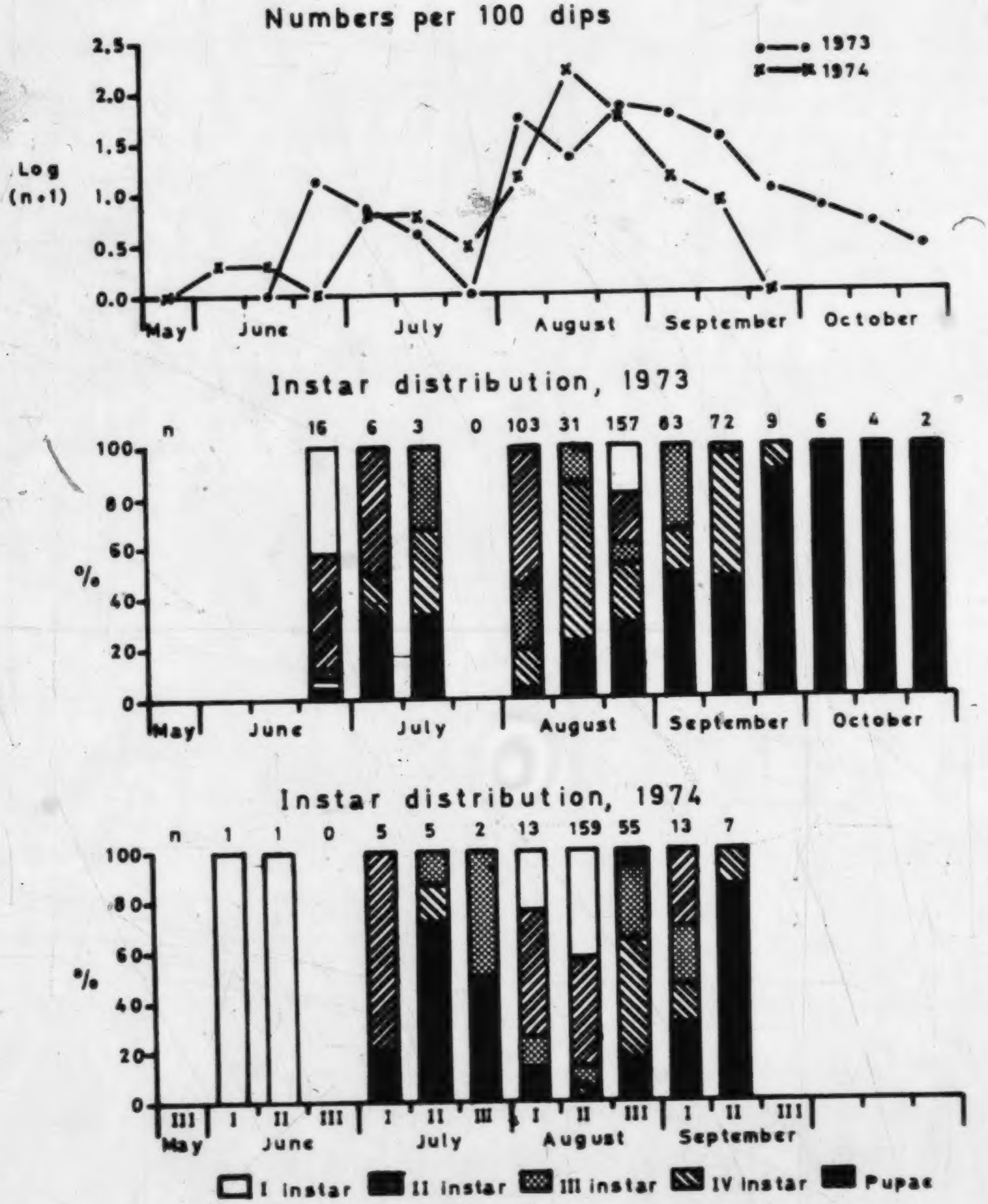


Fig. 36. Seasonal abundance and instar distribution of *Culiseta inornata* larvae and pupae in the lakeside pond.

from *Cs. inornata* in California. The infection rate in 1973 was 4 of 290 larvae collected in August and September (1.4%), and detection of infected larvae in this season was favoured by the practice of rearing them in the laboratory. No infections were seen in any of the 62 IVth instar larvae collected in 1974.

3.12.4. Blood feeding habits

Of the 87 blood meals identified (Table 17), 80 were from bovines (92%). The high proportion of bovine feeds at George Lake was not surprising since the box shelters from which most feds were obtained were beside a pasture, the New Jersey trap was by a cattle trough, and the shed was in the farmyard. At Edmonton, however, as noted earlier, the nearest cattle were 2.5 km from the site of collection, (see Section 3.4.). The two passerine feds, collected on 25/vi and 10/viii, do not provide any evidence of a seasonal shift in host-preference. Tempelis (1975) in his review states that studies of the feeding habits of *Cs. inornata* in several areas in North America indicate an overwhelming preference for bovines. In southern Alberta Shemanchuk, Downe and Burgess (1963) found that 74% of the positively identified *Cs. inornata* had fed on cattle, but other hosts included pig, sheep, horse, human, rodent, dog and fowl, none exceeding 6 %. These figures allow for a misprint in the paper, (J. Shemanchuk, personal communication).

Without information on the biting cycle of *Cs. inornata* throughout the diel, we cannot be sure that the females were not biting cattle earlier or later in the year then they were seen, but at some

Table 17. Blood meal identifications of *Culiseta inornata* females from George Lake and Edmonton, 1974-75.

	Bovine	Unidentified mammal	Passerine bird	Negative	Total
George Lake					
Box shelters	10	0	0	3	13
New Jersey Trap	24	2	0	9	35
Shed	1	0	0	0	1
Edmonton					
New Jersey Trap	0	0	0	1	1
Windows	45	3	2	3	53
TOTAL	80	5	2	16	103
% (of total positives)	92.0	5.7	2.3		

time other than the first hour after sunset. The 15 females collected on 10 - 11/vii/73 landed on me at intervals throughout the night, with no distinct peak time. This does at least demonstrate that *Cs. inornata* sometimes bites right through the night. Shemanchuk et al (1963) found that most of the unfed and fed *Cs. inornata* they caught in visual response traps in southern Alberta entered during the night with the main peak around midnight. During summer at George Lake *Cs. inornata* did not usually arrive at the calves until about ten minutes after sunset.

In the spring of 1975 one *Cs. inornata* was taken in late May in the hour before sunset, but no more were taken until late June and then they arrived in the first hour after sunset, (Fig. 26). The cessation of *Cs. inornata* attacks on cattle in late August was not because conditions in the first hour after sunset became inimical to feeding. Temperatures in early September were as high or higher than in early August when many *Cs. inornata* were taken, and *Aedes* were still taken in September, (Fig. 37).

3.12.5. Fecundity, egg retention and abnormal ovarian development

One hundred and one wild-caught females had a mean of 144.6 eggs, (range 72 - 273 , standard deviation 39.3). The distribution of egg batch sizes was skewed to the left, (Fig. 38). Individuals with less than 100 eggs were found only in July and August and may have been multipars. In *Anopheles maculipennis* such individuals produce fewer eggs, (Detinova, 1962). Other mean egg batch sizes reported for *Cs. inornata* are 140 for colony females, (Owen, 1942), and 204

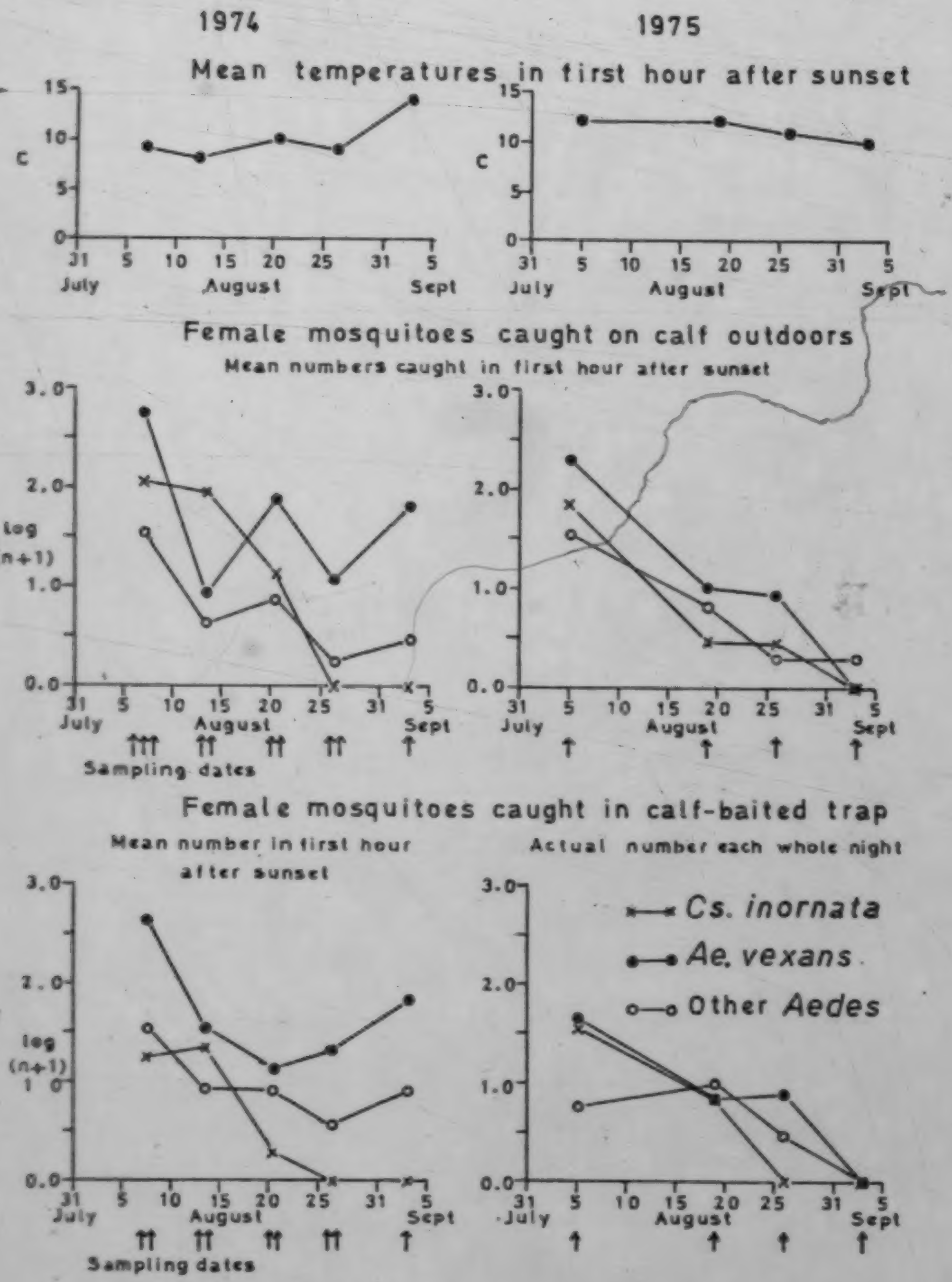


Fig. 37. Attack rates of *Cs. inornata* and *Aedes spp.* on cattle in August, in relation to temperature.

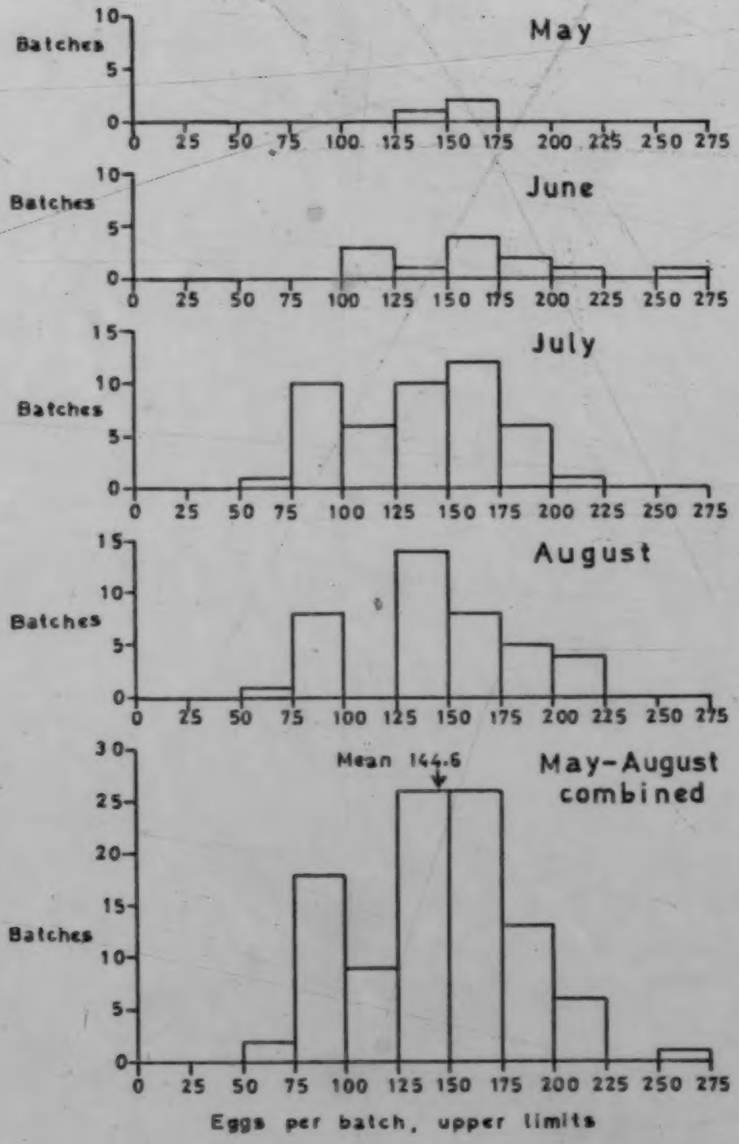


Fig. 38. Individual and seasonal variation in egg batch sizes of *Cs. inornata.*

for wild-caught females in Nebraska in early spring (Edmunds, 1958). Retained eggs were found in 144 of 1234 pars examined, (11.8%). The proportion of pars with retained eggs was highest in May, and throughout the season most of them only had one egg, (Table 18) but 3 females with 11 to 15 eggs were taken in July and August. They may have been older females that retained a few eggs from each batch.

A nullipar collected from a calf on 6/viii/74 showed uneven follicle development. All the follicles in one ovary were in stage N1, and most follicles in the other were in stage IIb, with a few in stages I, IIa and III. One of the two females collected from the calf on 26/viii/75, the last of the year, was apparently a normal gravid with 86 eggs. The number of eggs seems too large to be retained while seeking a fresh blood meal, and she may simply have been dazzled by the flashlight. Service (1969) found 6 gravids among 32,000 females (mostly *Aedes spp*.) collected from human bait. A female collected on 6/viii/74 from a calf had no blood visible, but laid 15 eggs 3 days later on moist filter paper on the floor of its holding cup. These were probably eggs from a previous gonotrophic cycle since it was too soon to have matured eggs from any blood taken on the day of capture.

3.12.6. Gonotrophic concordance of females in August

In 1974 the last engorged females collected in the calf-baited trap, 7 on 13/viii, 22 on 14/viii and 3 on 19/viii, were held for one week in a shaded, unheated box on the forest floor, then dissected. All showed normal egg development except for one

Table 18. Numbers of parous *Culiseta inornata* with retained eggs in each month, May to August.

		Number of retained eggs						
		0	1	2	3	5-10	11-15	Total
May	No.	26	8	2	0	0	0	36
	%	72.2	22.2	5.6	0	0	0	100.0
June	No.	288	22	6	2	3	0	321
	%	89.8	6.8	1.9	0.6	0.9	0	100.0
July	No.	491	49	9	5	3	2	559
	%	87.8	8.8	1.6	0.9	0.5	0.4	100.0
August	No.	284	23	7	1	1	1	317
	%	89.6	7.2	2.2	0.3	0.3	0.3	99.9
Total	No.	1089	102	24	8	7	3	1234
	%	88.2	8.3	1.9	0.6	0.6	0.3	100.0

which had a mosaic of follicles in stages II and III, too far advanced to be in reproductive diapause. This evidence is against the idea of late summer gonotrophic dissociation by *Cs. inornata*.

3.12.7. Autogeny in laboratory-reared females

Seven cases of autogenous follicle development were seen in laboratory-reared females from Edmonton stocks that had been colonised for 2 - 26 months (2 to approximately 18 generations). Autogeny was not common, and did not appear in one of the two experiments (autogeny 2), designed to measure its frequency, (Table 19). Four of the seven cases were found in the diapause induction experiments, described fully in Chapter 6. The total of 291 females includes only those that were never offered blood, and were at least 14 days old when dissected. Four of the dissected females (1.4 %) had follicles in stages III - V. If like Corbet (1967) one only considers follicle development to stage V to be true autogeny, the number of cases falls to 2 (0.7 %). Moreover, in autogenous *Culex tarsalis* from Nevada eggs were laid as early as 5 days after emergence, (Chapman, 1962). If I had included the number of females that were 5 - 13 days old when dissected the autogeny rate would have been reduced still further.

In the autogeny 1 experiment only two small egg rafts were laid by the parental generation (stock from a colony that had been kept for 26 months in the laboratory), and the F_1 larval mortality was high. Only one small raft was produced by the 8 F_1 females and the 3 larvae that hatched from it all died. In all the experiments the larvae were fed a 2:1 mixture of powdered rabbit pellets and

Table 19. Summary of records of autogeny in laboratory colonies of *Cs. inornata* from Edmonton stock.

(a) Autogeny revealed by dissection.

Experiment	Age (days)	No. diss.	Autogenous No.	Autogenous %	Stage	No. eggs
Diapause 1[(x)]	15-25	16	1	6	IV	49
Diapause 2	14-17	51	1	2	III	52
Diapause 3	14-15	100	2	2	1.V	37
					2.V	6
					III	4
Autogeny 2	26-29	124	0	0	-	-
Total		291	4	1.4		

(b) Autogeny revealed by oviposition (autogeny experiment 1).

Generation	No. of females[(y)]	Age (days)	Egg rafts No.	Egg rafts %	Numbers of Eggs	Numbers of Larvae	Numbers of Adults
Parental	96	16-25	2	2	1. 23	20	13
		30-39			2. 32	7	0
F_1	8	27-30	1	12	31	3	0

(x) Details of diapause induction experiments 1-3 given in Chapter 6.

(y) 110 females in parent generation but only 96 survived more than 14 days.

bakers' or brewers' yeast and the adults were given soaked raisins. Spielman (1971) states that while genetically anautogenous mosquitoes cannot be made autogenous by superabundant larval feeding, autogenous development can be suppressed by the starving or overcrowding of genetically autogenous larvae. The larval diet I supplied was adequate for maintaining the colony for two years when the adults had blood, but one cannot be sure that the autogeny rate would not have been increased by a different larval diet.

Owen (1942) reported that he saw only three autogenous females in a colony of *Cs. inornata* from Wyoming, (total number not stated), and none of them laid more than 15 eggs. Washino and Shad-Del (1962) found that 4 of 27 females (15 %) from larvae and pupae collected in California in April developed some of their follicles to stage III or beyond. These two authors mistakenly state that Chapman (1962) found autogeny in *Cs. inornata* in Nevada. He did not examine any.

Two possible cases of autogeny were found in dissections of wild-caught, unfed females. One was caught in the New Jersey trap at George Lake on 16/vii/74 and had all her follicles in stage III. The other, a nullipar taken from cattle on 22/vii/74, had 2 follicles in stage III and the rest in stage Ib. If these were autogenous females they were only 2 out of 882 nullipars collected (0.25 %) during the gonoactive season (here taken as early May to mid-August). Both might have taken small blood meals before.

The mean number of follicles at stage III or beyond in the 7 laboratory cases was only 33.4. Compared with the autogeny of a

Culex tarsalis population in California, in which over 95 % of the wild-caught females in July and August are autogenous and produce an average of 90 eggs, (Spadoni, Nelson and Reeves, 1974), autogeny in local *Cs. inornata* does not seem to be an important factor in their reproduction. However, most of the first females to appear in the spring were parous, and this has been used as evidence for autogeny in *Mansonia richiardii* in England (Service, 1969) and for several Tabanid species in Alberta, (Thomas, 1972). It may be that only the overwintered females are autogenous.

3.12.8. The teneral stage

Only 20 tenerals were collected, 16 of them in box shelters and in the Stevenson screen close to the lakeside pond, a known breeding site. All but one of the tenerals (95 %) were inseminated (Table 20), a much higher rate than in the other species (0 - 12 %). This may be because most females become inseminated at the site of emergence. Carpenter observed copulating pairs at the edge of a pool (Carpenter and LaCasse, 1955). On 24/viii/72 I observed many males resting on the mossy banks in a shady part of the lakeside pond. They made short flights over the surface of the pond, touching the water surface every 15 cm or so, then returned to the bank and rested for about 30 seconds before making another flight. Copulation does not only occur at the emergence site; a couple was taken on one of the windows on 3/ix/75.

The follicles had reached stage I in 44 % of the tenerals, and 70 % had fatbodies rated 1, but only one teneral (5 %) had syrup

Table 20. Insemination rates, follicle and fatbody development and presence of meconium in teneral and post-teneral *Culiseta inornata*.

		Teneral (with muscle remnants)	Post-teneral (without muscle remnants)
No. examined		20	1489
% inseminated		95	98.3
No. examined		18	2129[a]
% in stage	N1	0	0.3
	N2	56	1.5
	I	44	71.0
	IIa	0	19.6
	IIb	0	7.6
No. examined		20	3521
% with fatbody rated	0	30	4.6
	1	70	29.4
	2	0	53.0
	3	0	13.0
No. examined		20	3549
% with meconium		10.0	0.1
Number examined		20	1592
% with syrup in crops		5	52.2

(a) Post-teneral nullipars only.

in her crop. Meconium was found in 10 % of the tenerals and in 0.1 % of the post-tenerals.

3.12.9. Follicle development

Most nullipars at bait had F:G ratios greater than 2.0 and follicles in stage II, (Fig. 39), but most pars, both at bait and at other sites, had follicles in stage I, and a quarter of them had F:G ratios of 1.6 - 2.0. The nullipars at sites other than bait were the most diverse group owing to the presence of both gonoactive and diapausing individuals, and seasonal changes in the distribution of F:G ratios in this group are shown in Fig. 40. From early July to early August most nullipars had F:G ratios greater than 2.0, but from mid-August onwards most had F:G ratios of less than 1.5. This was the time when numbers at bait sharply declined and feds and gravids ceased to appear at other sites. Setting an upper limit of 1.5 for the F:G ratio females in diapause agrees with the seasonal feeding pattern, except for a few apparently diapausing females in July and early August, and a few apparently gonoactive females in September and October. Using this upper limit the percentages of nullipars in diapause in 1974 and 1975 are shown in Fig. 41. In both years the major increase in diapausing females occurred in mid-August. The rather abrupt changes in October may have beendue to the small sample sizes.

In May, June and early July most nullipars at sites other than bait had follicles in stage I, (Fig. 42). From mid-July to early August most had follicles in stage II, and from mid-August onwards

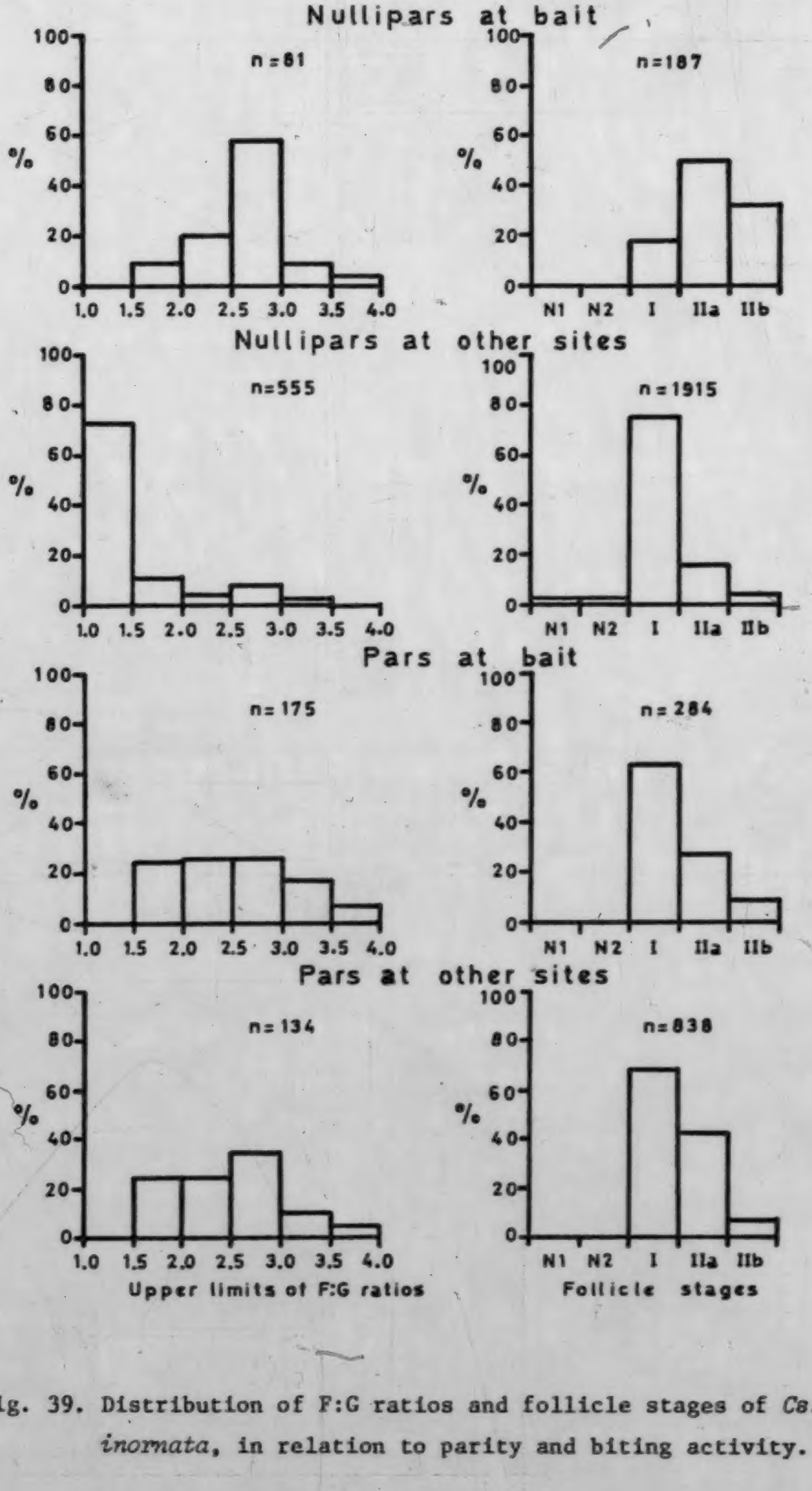


Fig. 39. Distribution of F:G ratios and follicle stages of *Cs. inornata*, in relation to parity and biting activity.

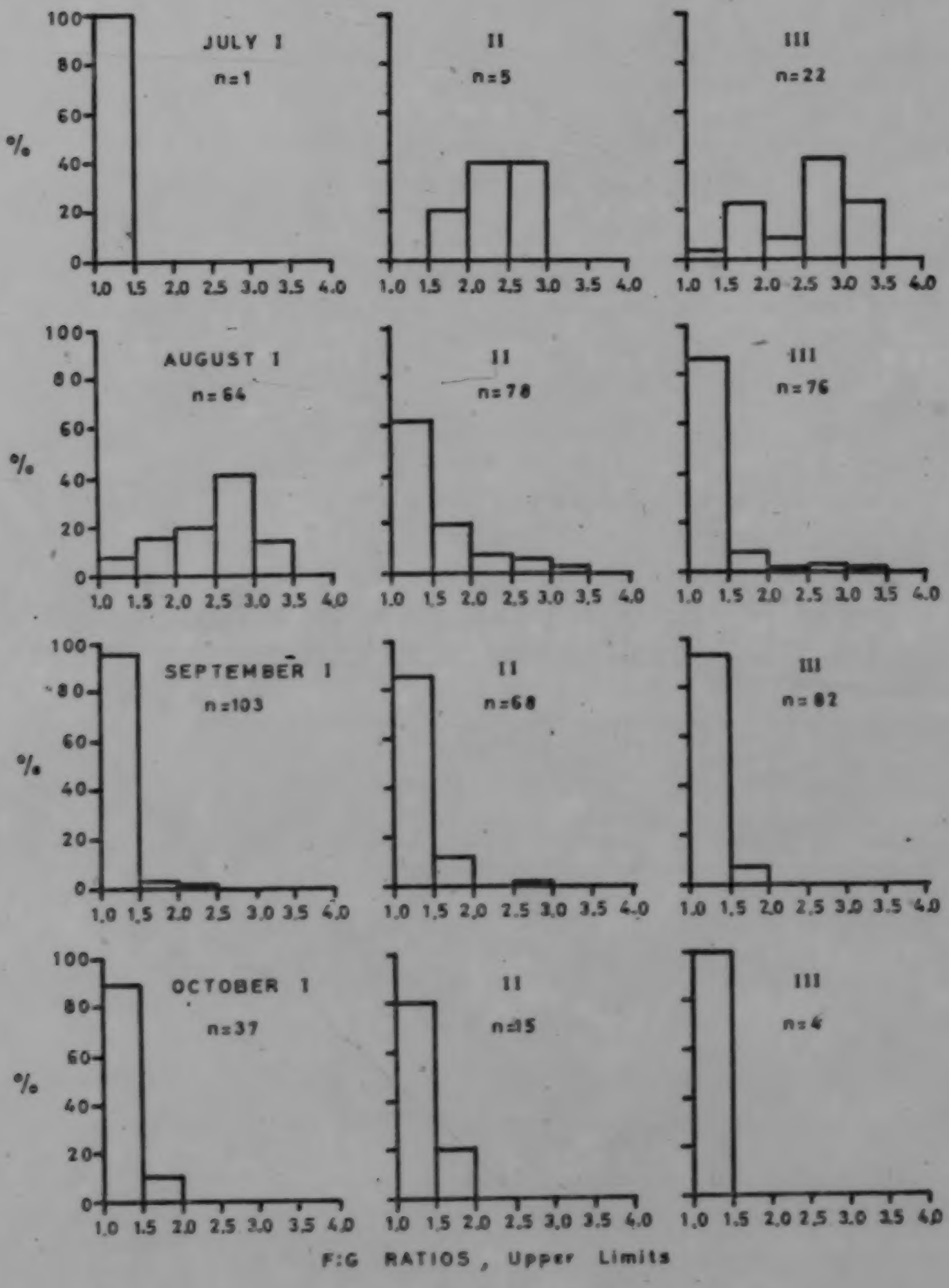


Fig. 40. Seasonal changes in distribution of F:G ratios of nulliparous *Cs. inornata*. From sites other than bait.

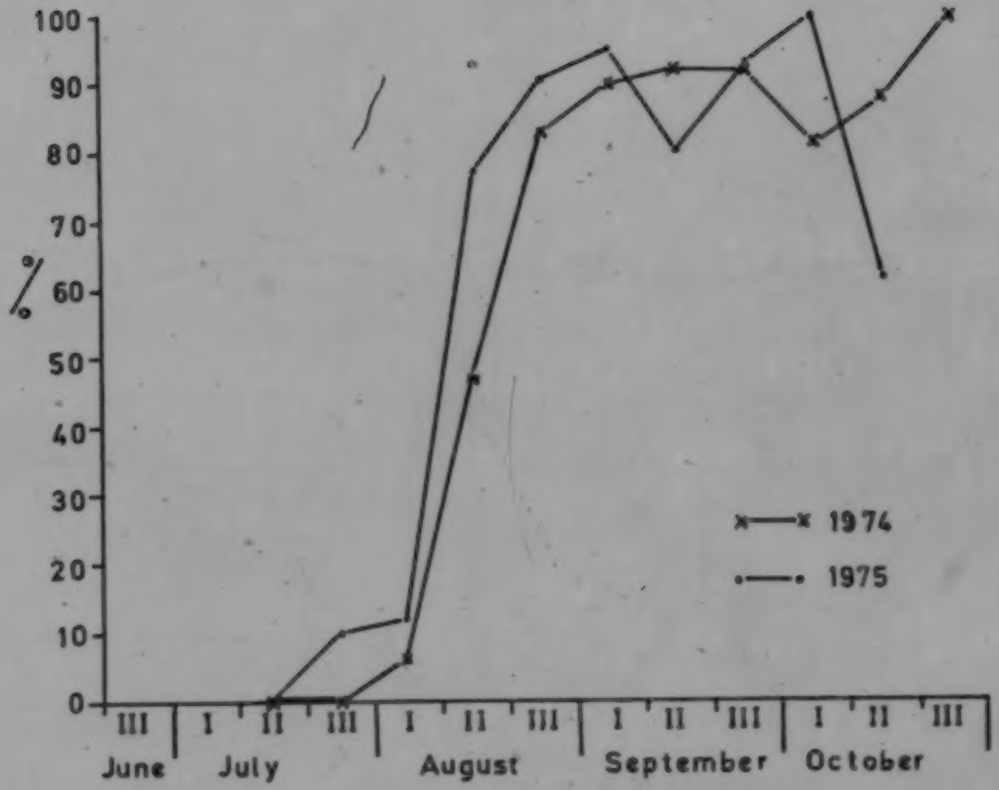


Fig. 41. Diapause rates in nulliparous *Cs. inornata*, 1974 and 1975.

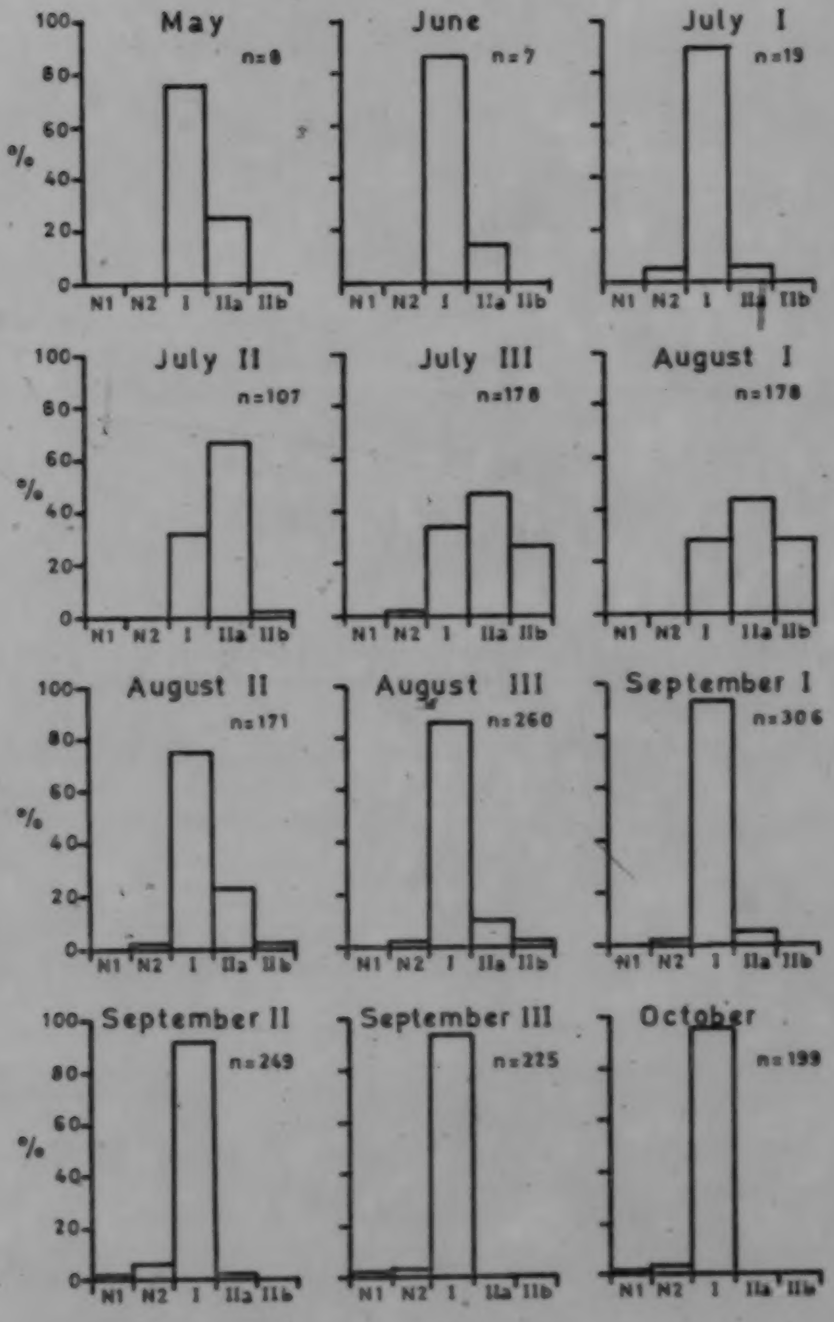


Fig. 42. Seasonal changes in distribution of follicle stages of nulliparous *Cs. inornata*. From sites other than bait.

most were in stage I, though a few with stage II follicles were found up to mid-September. Most nullipars had follicles in stage II during the period when peak numbers were taken at bait. This association between gonoactivity and the possession of stage II follicles was not seen in May, but the sample was small.

3.12.10. Fatbody development and fungous infections

More fat females were taken at other sites than at bait, and more fat nullipars than pars (Fig. 43). Amongst the nullipars, the diapausing females (segregated by F:G ratio) were not fatter than the non-diapausing ones. The main difference in fatbody ratings was a seasonal one. From July onwards, when many of the Compositae flowered, (see section on flower visiting), the proportion of females with abdomens filled or distended with fatbody rose steadily in all groups.

In August, September, and October, 35 unfed, nulliparous females from the New Jersey traps, windows, a *Tanacetum* flower and the *Carex* meadow were found with their abdomens infected with fungus. Seasonal infection rates are shown in Table 21. The numbers dissected include only nullipars at sites other than bait, since all infected females fell in this group. Most infected females seemed to be in ovarian diapause. The earliest was taken on 9/viii and the latest on 23/x. The abdomens of infected females were distended with fatbody but it was loose and crumbly and seemed to be the main site of infection. The basic unit of the fungus was an oval structure about 50 µm long with granular contents. These units branched or budded and the mycelium resembled that of *Coelomomyces pentangulatus*, as

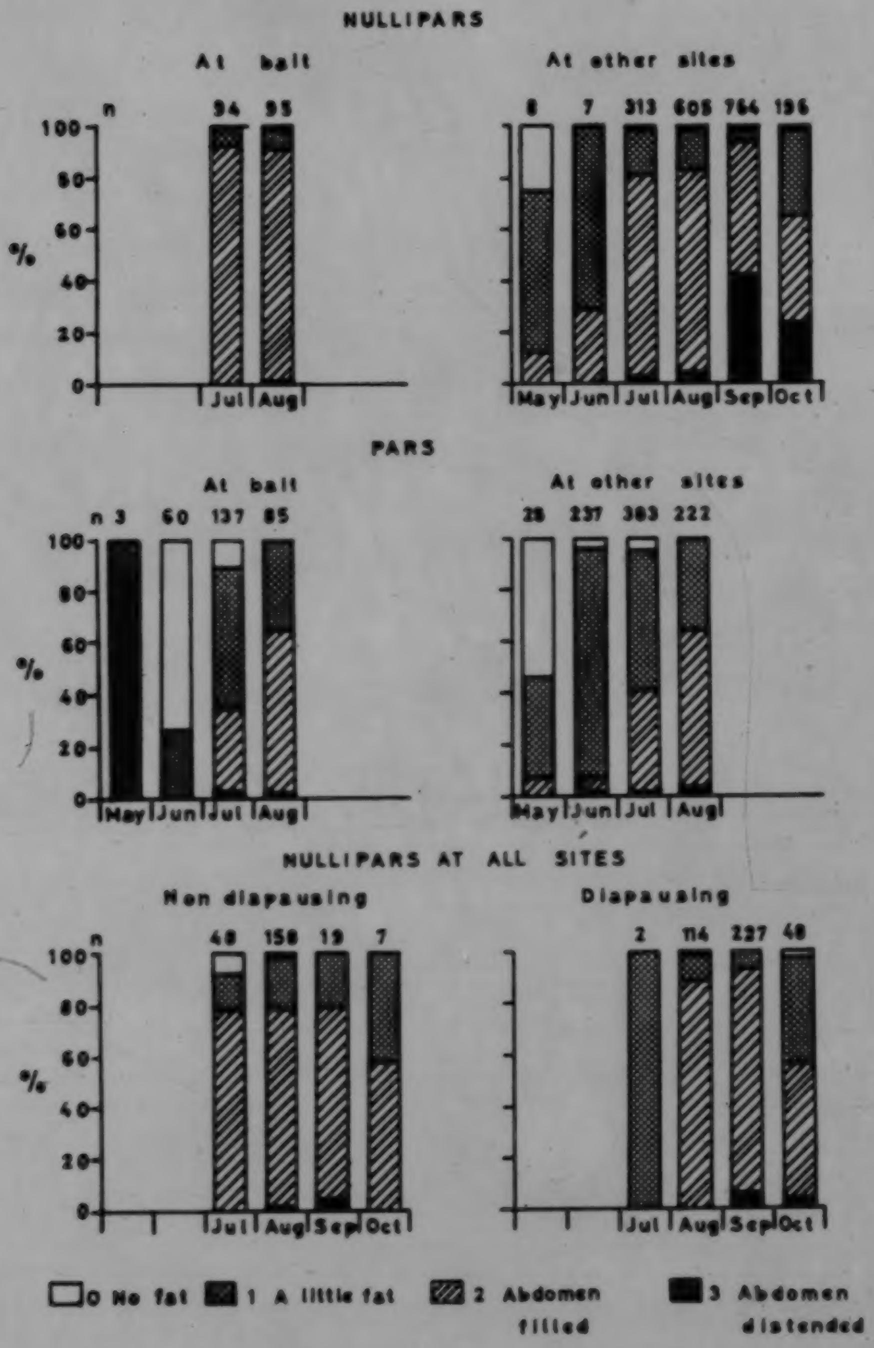


Fig. 43. Seasonal changes in fatbody ratings of *Cs. inornata* in relation to parity and biting activity.

Table 21. Seasonal fungous infection rates of *Culiseta inornata* females (nullipars at sites other than bait).

		1973		1974		1975		All years		
		No. exam.	No. inf.	No. exam.	No. inf.	No. exam.	No. inf.	No. exam.	No. inf.	% inf.
August	I	87	4	68	0	27	0	182	4	2.2
	II	84	1	40	1	52	1	176	3	1.7
	III	134	6	49	1	44	0	227	7	3.1
September	I	132	0	60	2	48	0	240	2	0.8
	II	135	0	40	4	34	1	209	5	2.4
	III	136	0	59	5	31	3	226	8	3.5
October	I	36	0	21	1	16	2	73	3	4.1
	II	89	0	10	1	6	1	105	2	1.9
	III	3	0	4	1	0	0	7	1	14.3
TOTAL		836	11	351	16	258	8	1445	35	

NB No infected females among 324 dissected in July.

figured by Couch and Umphlett, (1963).

Twelve infected abdomens were examined by C. J. Umphlett, Botany Department, Clemson University, South Carolina, however, and his diagnosis was "possibly conidia of an imperfect fungus and mycelium of a phycomycete (not *Lagenidium giganteum*)". All the infected females except possibly the one collected from the *Carex* meadow, were well enough to fly to the sites were they were collected, but any infection destroying or damaging the fatbody would reduce the chances of overwintering successfully.

3.12.11. Crop contents

Although the fatbody in *Cs, inornata* was not so well-developed as in some other species, such as *An. earlei*, more female *Cs. inornata* had syrup in their crops than in any other species. Nullipars more often had syrup than did pars, and those at bait more often than those at other sites, (Table 22). The highest rate, 79 %, was seen in early September, and 50 % of those caught in mid-October, after most flowers were over, had syrup. Bacteria-like rod-shaped bodies were found in the syrup in five individuals, 2 in July, 1 in mid-August, and 2 in late August, 1974. Thus syrup might have come from other sources than flowers, such as rotting fruit or aphid honeydew . Mosquitoes in Maryland have been observed feeding on damaged or overripe fruits such as grapes (Joseph, 1970).

3.12.12. Summary of seasonal development

Although *Cs. inornata* was not found in winter, the observed seasonal development (Fig. 44) suggests that it is adapted for

Table 22. Seasonal changes in numbers of *Culiseta inornata* females with syrup in their crops, in relation to parity and biting activity.

	Nullipars						Pars					
	At bait			At other sites			At bait			At other sites		
	Diss no.	Syrup no.	%	Diss no.	Syrup no.	%	Diss no.	Syrup no.	%	Diss no.	Syrup no.	%
May III	0	-	-	0	-	-	3	0	0	0	-	-
June II	0	-	-	1	0	0	0	-	-	0	-	-
III	0	-	-	0	-	-	52	9	17	45	16	36
July I	0	-	-	1	0	0	30	10	33	44	11	25
II	2	0	0	13	7	54	35	9	26	73	30	41
III	62	29	47	110	75	68	45	18	40	31	18	58
August I	42	28	67	94	57	61	24	17	71	83	43	52
II	30	18	60	90	46	51	32	19	59	31	10	32
III	8	4	50	92	39	42	7	2	29	8	5	62
September I	0	-	-	108	85	79	0	-	-	0	-	-
II	0	-	-	73	54	74	0	-	-	0	-	-
III	0	-	-	90	65	72	0	-	-	0	-	-
October I	0	-	-	37	21	57	0	-	-	0	-	-
II	0	-	-	16	8	50	0	-	-	0	-	-
III	0	-	-	4	1	25	0	-	-	0	-	-
Total	144	79	44.8	729	458	62.8	228	84	36.8	315	133	42.2

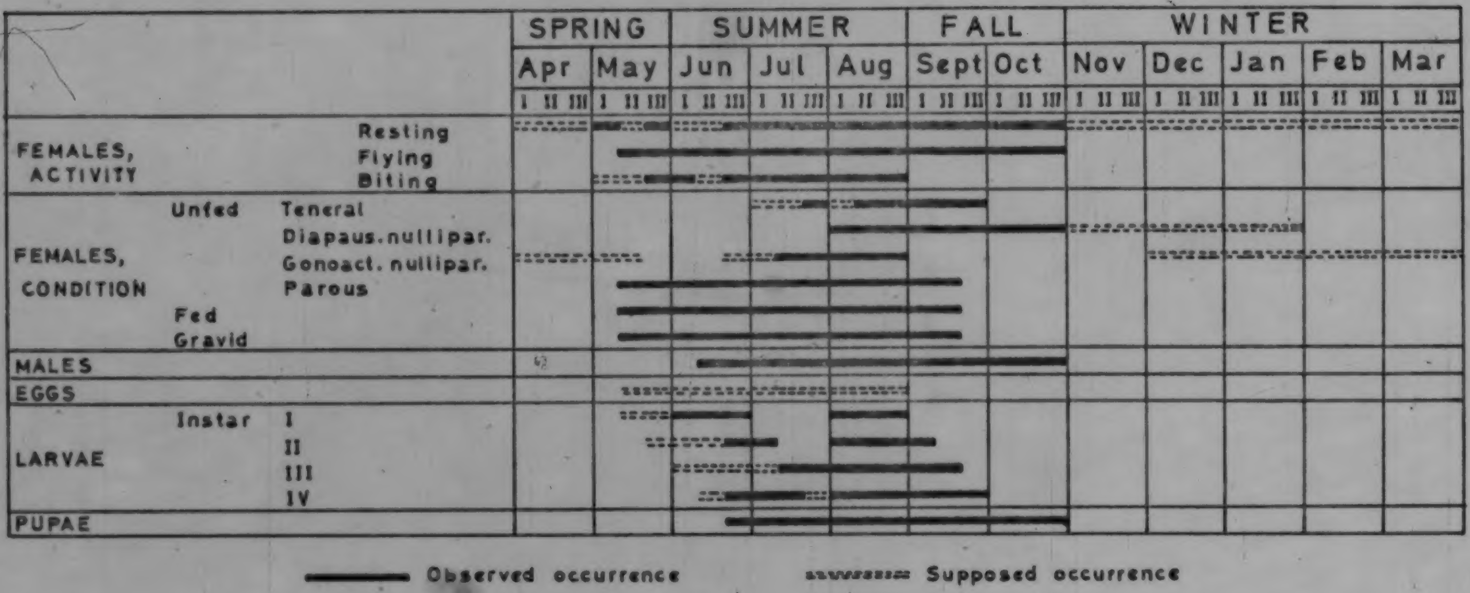


Fig. 44. Summary of seasonal biology of *Cs. inornata*.

overwintering in the adult stage. The earliest individuals collected in Spring were adult females and the last collected in fall were pupae and adults. If overwintering were in the larval stage one would expect an arrest of larval development in the fall at a particular instar, but as fall progressed the larvae disappeared and only pupae were taken. If overwintering were in the egg, one would expect females in the fall to continue blood feeding and producing more eggs,but all the females were in diapause. Although eggs or larvae might sometimes survive the winter, it does not seem to be part of the strategy of the local race.

3.13. *Culiseta morsitans dyari*

The first females of this subspecies that I collected I identified as *Culiseta incidens* because of a tendency for the wing scales to be grouped more densely in some patches, and I used this name in field notes, computer analysis of dissections, and several reports, one of them published, (Hudson, 1974). On reexamination of pinned adults (47 females and 65 males) I would assign all of them to *Cs. m. dyari*, except for 3 female *Cs. s. minnesotae* and 2 male *Cs. impatiens*, and none to *Cs. incidens*.

The data on seasonal abundance of adults, (Fig. 45), are hard to interpret because few adults were collected. Males and females were taken from mid-June to mid-October, the best sources being the windows, New Jersey traps and box shelters, in that order. (In Fig. 45, note that the four graphs are not all to the same scale). The first females in the quail-baited traps were in late July (24/vii), though the traps

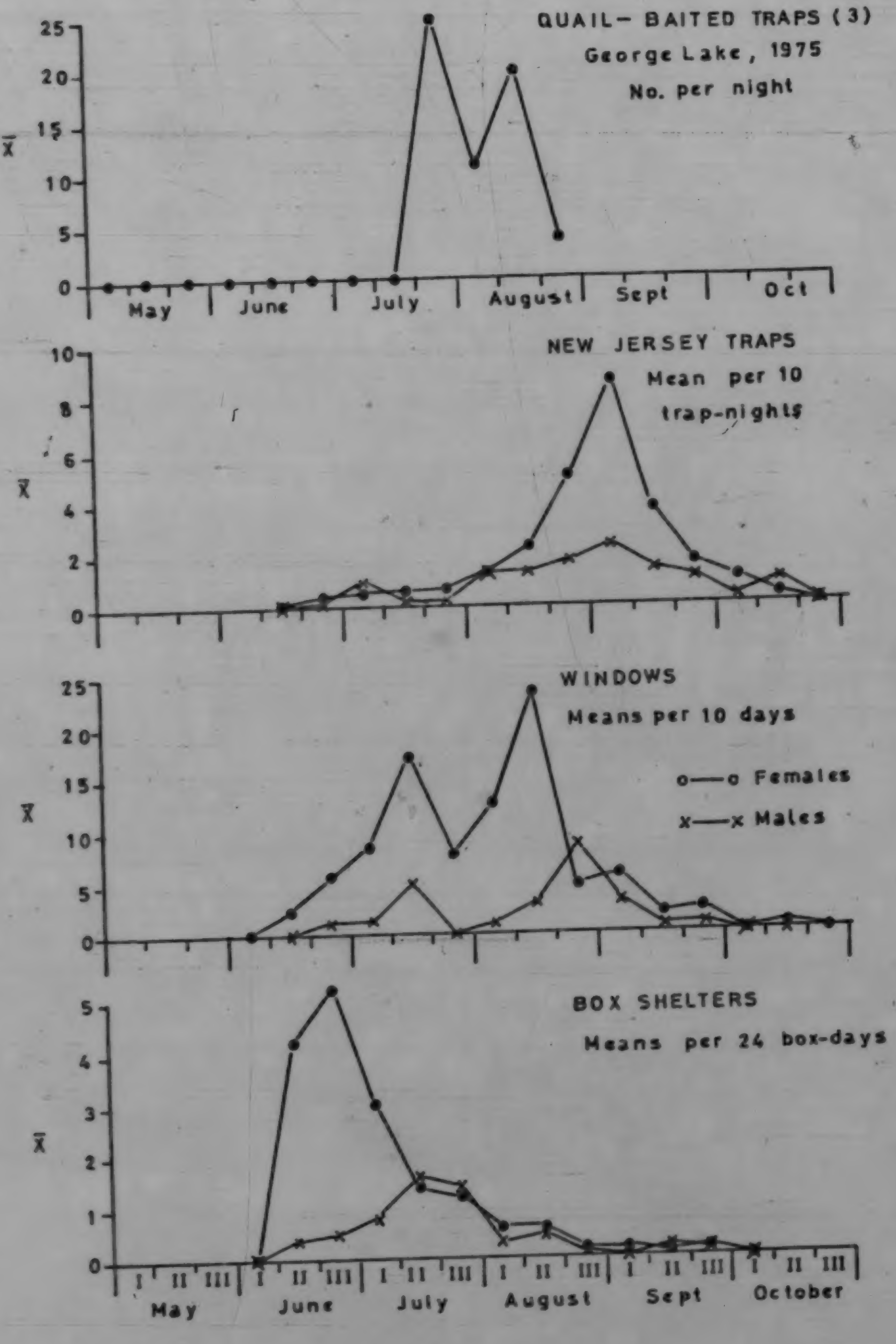


Fig. 45. Seasonal abundance of *Culiseta morsitans dyari*.

had been out once each decade from early May. Four females were taken the last night the traps were used, (26 - 27/viii). The other three collecting methods disagree on the period of maximum abundance. The box shelters suggest that it was late June, the windows mid-August, and the New Jersey traps early September.

Blood-feds were taken from late June to mid-October and gravids from early July to late September (Fig. 46), over a month later than in any of the other species. The first pars were taken in mid-July; from mid-August onwards, most were parous. The data do not indicate the number of generations per season. Nullipars were taken in October, and females would have been first instar larvae no earlier than August. If overwintering is in the egg, these last nullipars could have come from eggs laid the previous year which did not hatch until August, either because they were not flooded before, or because more than one flooding was needed to hatch them. Alternatively there could be a facultative egg diapause, those eggs laid before a certain date hatching immediately and developing to adults the same year, and those eggs laid after that date not hatching until the next year. Egg hatch after early August would not allow the resulting adults time to feed and produce eggs before winter.

A female *Cs. m. dyari* was reared from a group of *Culicella* larvae collected from the lakeside pond on 30/viii/73; all the others were *Cs. s. minnesotae*. A IV instar *Culicella* larva was collected by City of Edmonton staff c.13/v/75 at Sherwood Park, 11 km east of the city. The antennae and most setae were missing and the larva could have been either *Cs. m. dyari* or *Cs. s. minnesotae* but the early

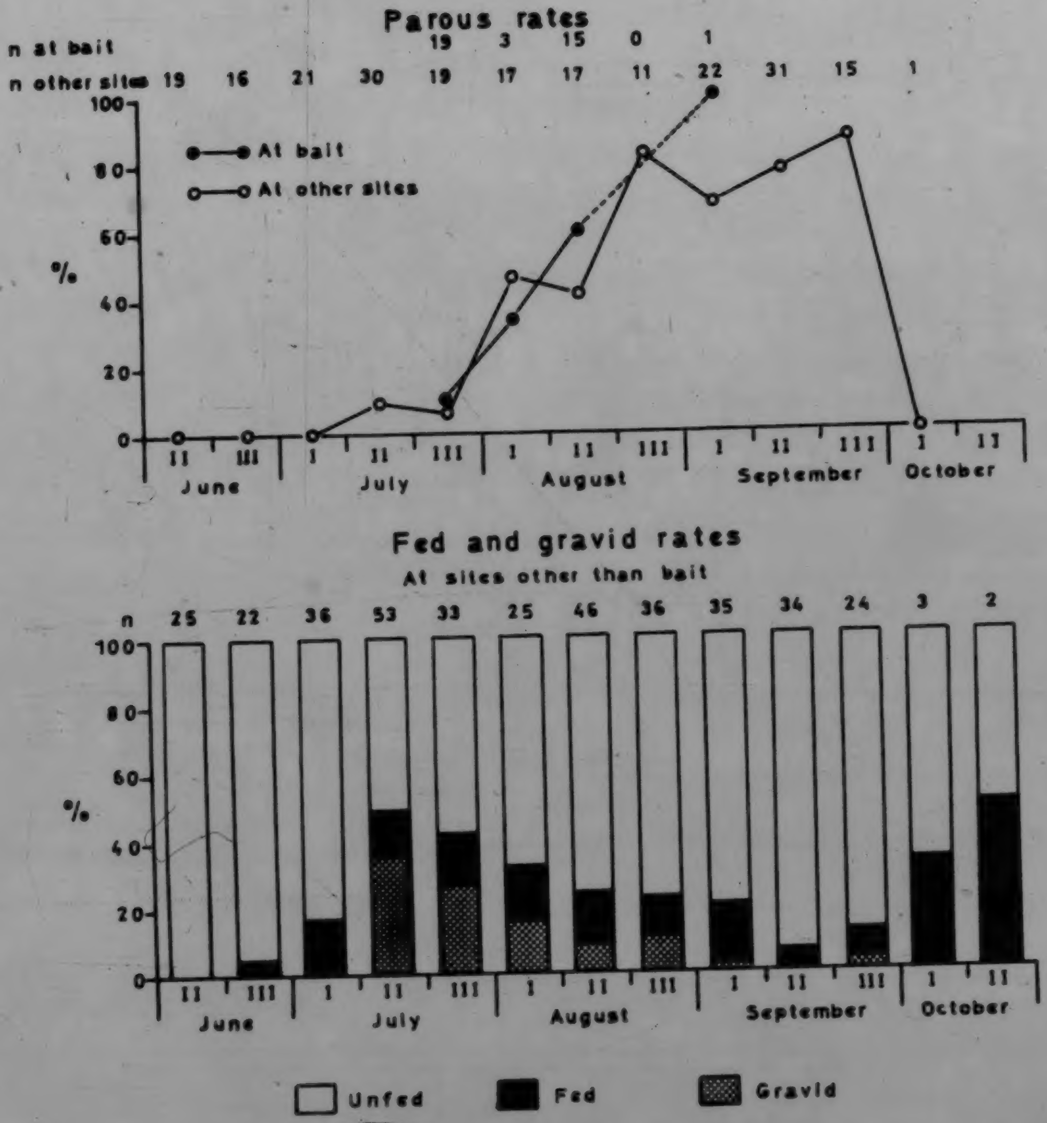


Fig. 46. Seasonal changes in fed, gravid and parous rates of *Cs. m. dyari*.

date suggests the former.

Of 29 blood meals tested, 22 from Edmonton and 7 from George Lake, 28 reacted. Fourteen of the positives were passerine (50 %), 2 hawk (7 %) and 12 "unidentified bird" (43 %). Preference for Passerines is not proven because more than half the bird species and probably most of the individuals at both study sites belong to this order. Further evidence of attraction to birds was the high proportion of *Cs. m. dyari* in the quail-baited traps (24.7 %), though only 5 of the 60 collected (8 %) were engorged. A female was caught from human bait one hour after sunset on 23/viii/72, another (unfed) was collected in the calf-baited trap on 27/viii/74, and a third on a calf outside on 3/ix/74. Service (1971) reported that 95 % of *Cs. morsitans* tested from England had fed on birds, and a few on man, rabbit, bovid, and reptile.

Sixteen gravid females had a mean of 110.8 eggs, (range 54 - 194, standard deviation 37.7). This is very close to the mean of 109.2 ± 25.0 obtained for the English form, (Service, 1968a). Retained eggs were found in 15 of 93 pars (16 %); 10 had 1, 2 had 2, 2 had 3 and one had 11. One female laid eggs in a cluster on moist filter paper in the laboratory. The eggs did not hatch after more than a week at 20 C. They were rounded at one end and pointed at the other as figured by Marshall, (1938, Fig. 57u).

No teneral females were found, but 1 of 279 (0.4 %) had meconium, 38 of 269 (14.1 %) were uninseminated and 2 of 279 (0.8 %)

had follicles in stage N1. Most nullipars at bait had follicles in stage I and 24 % of them had F:G ratios of less than 2.0 (Fig. 47), but most pars at bait had follicles in stages IIa and IIb, and all had F:G ratios of more than 2.0. By contrast, most nulliparous *Cs. inornata* at bait had follicles in stage II and most pars at bait had follicles in stage I, (Fig. 39).

Females with F:G ratios of less than 1.5 were found only from mid-June to early August, and they were in the majority only in early July, (Fig. 48). These females with small follicles may have been recent post-tenerals, but no tenerals were found with them. Although the first blood-feds were taken in late June, (Fig. 46), blood feeding seems to develop quite slowly in the population. The quail-baited traps did not catch any females until late July, (Fig. 45), and the proportion parous did not reach 50 % until mid-August. It may be that *Cs. m. dyari* has a diapause 2 - 4 weeks after emergence before it takes blood, This time would be well spent feeding on flowers, which would be at their peak, and the reserves accumulated would help maintain the gonoactivity of females in late summer and fall. There could also be some advantage in laying eggs late, when pool sizes had stabilized and egg predators were less active.

Fatbody development was greatest in July, except for a late August sample of only two individuals (Fig. 49), and the females in September were thin. The most females with syrup in their crops were found in late June and early July, except for nullipars in mid-September, another small sample. These findings give some support, limited by small sample sizes, to the idea of a summer diapause

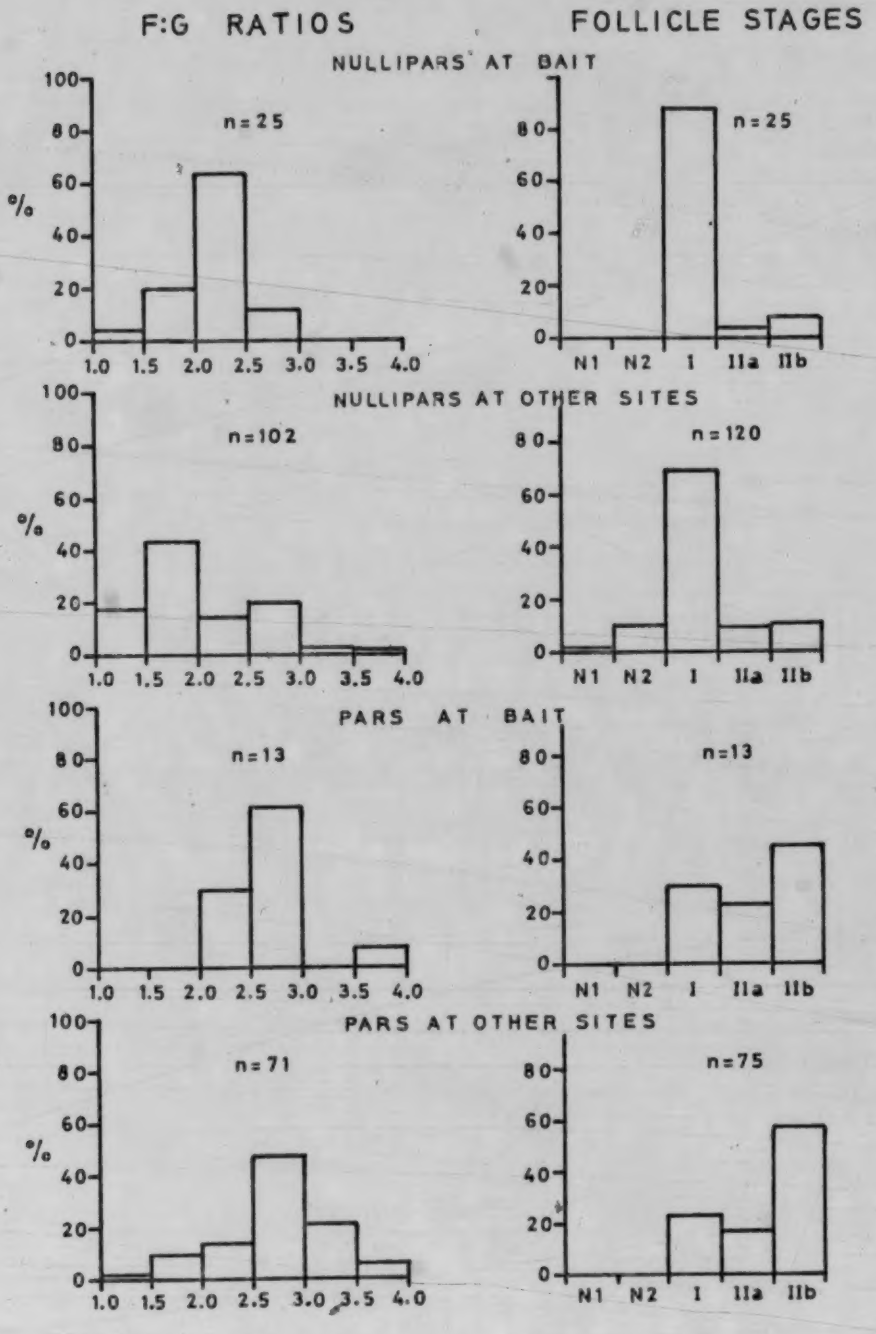


Fig. 47. Distribution of F:G ratios and follicle stages of *Cs. m. dyari* in relation to parity and biting activity.

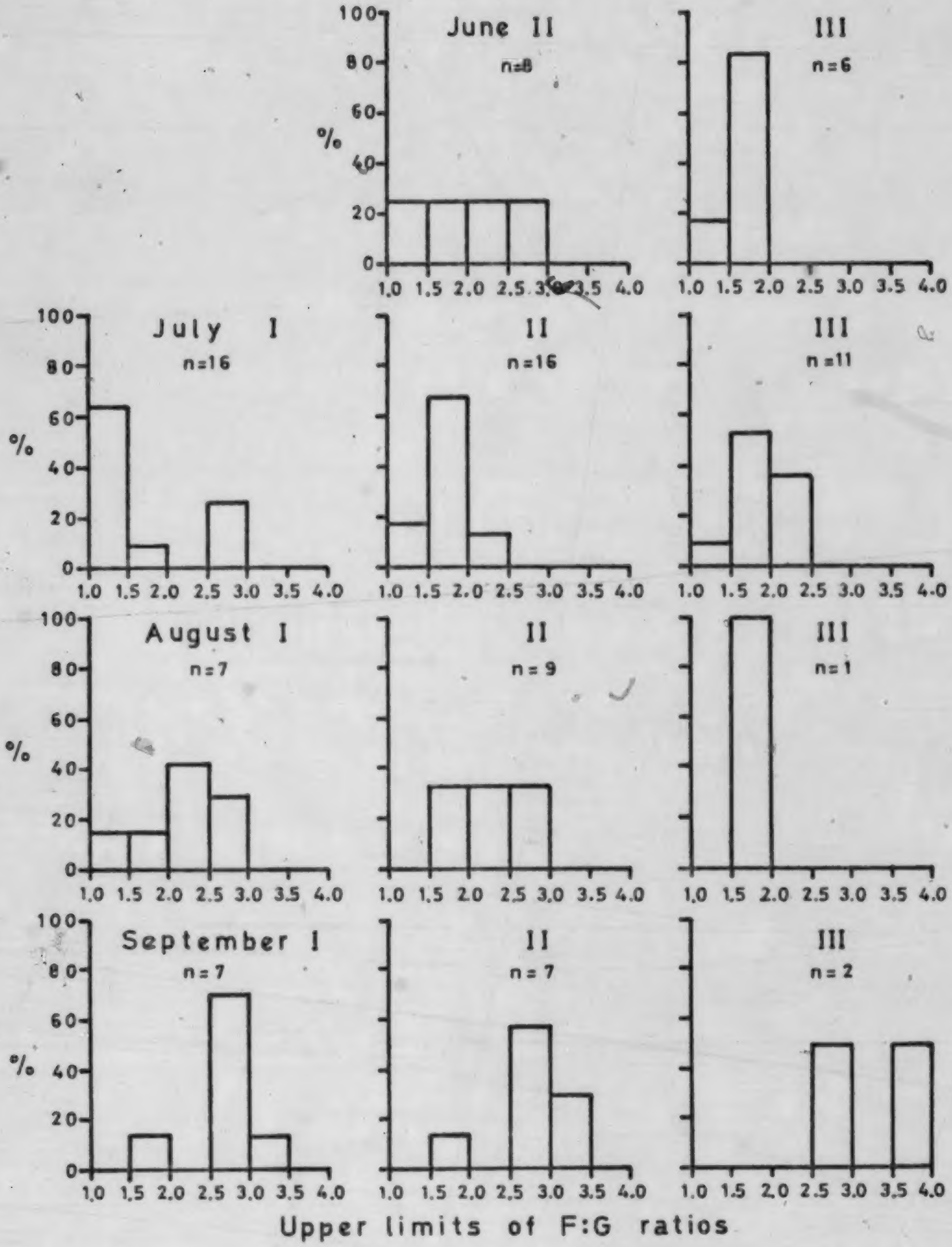


Fig. 48. Seasonal changes in distribution of F:G ratios of nulliparous *Cs. m. dyari*. From sites other than bait.

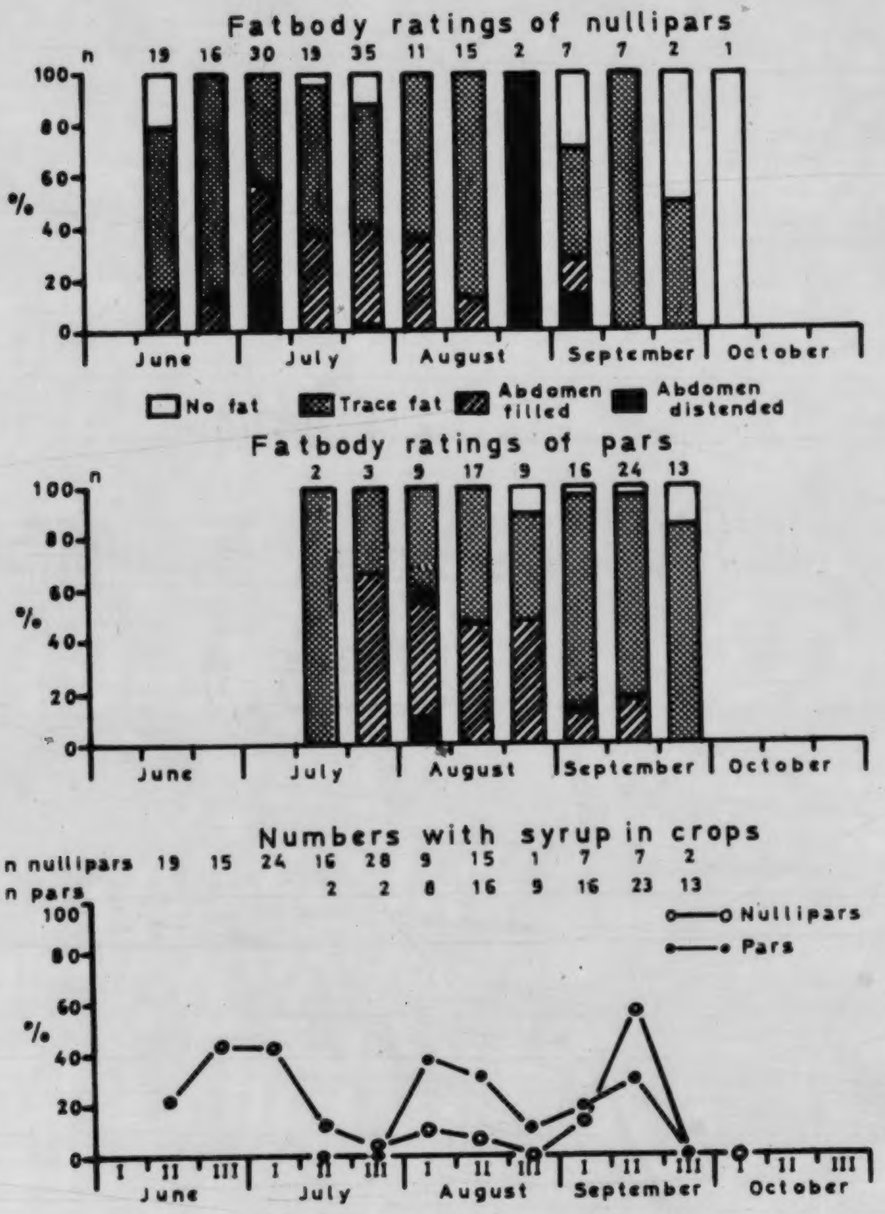


Fig. 49. Seasonal changes in fatbody ratings and crop contents of nulliparous and parous *Cs. m. dyari*.

favouring nectar feeding and fatbody accumulation.

Male swarms were seen on two evenings in 1974, while females of other species were being caught from a calf in the SE corner of a pasture, near the farmyard at George Lake. The first swarm was observed on 13/viii between 21:15 and 21:50 hours (mountain daylight time), or 0.20 to 1.05 crep, and had dispersed by 21:55. The temperature was 6 - 7 C. About 20 - 50 males were swarming 1.5 - 2.5 m over the edge of a patch of grass about 0.7 m high by the driveway, outside the fence. A few males were smaller than the rest. Four large and one small male captured were *Cs. m. dyari* and *Clx. territans*, respectively. The second swarm was seen [illegible] 26/viii, between 21:00 and 21:10 only, or 0.58 - 0.84 crep. The temperature was 10 - 11 C. About 60 males were observed swarming in almost the same place as before, about 2 m behind the tethered calf and 1.5 - 3.0 m above the ground. Three males were caught, all *Cs. m. dyari*. A female was found in the nearby calf-baited trap the next day. Both swarms were "marker swarms" in the sense of Nielsen and Haeger (1960), who do not give any previous records of swarming in *Cs. morsitans* (sens.lat.).

The seasonal pattern (Fig. 50) is incomplete because only one larva and no tenerals were found. The first appearance of adults in mid-June suggest overwintering as eggs rather than mature larvae, since overwintering in the III or IV instar should have permitted adult emergence in May.

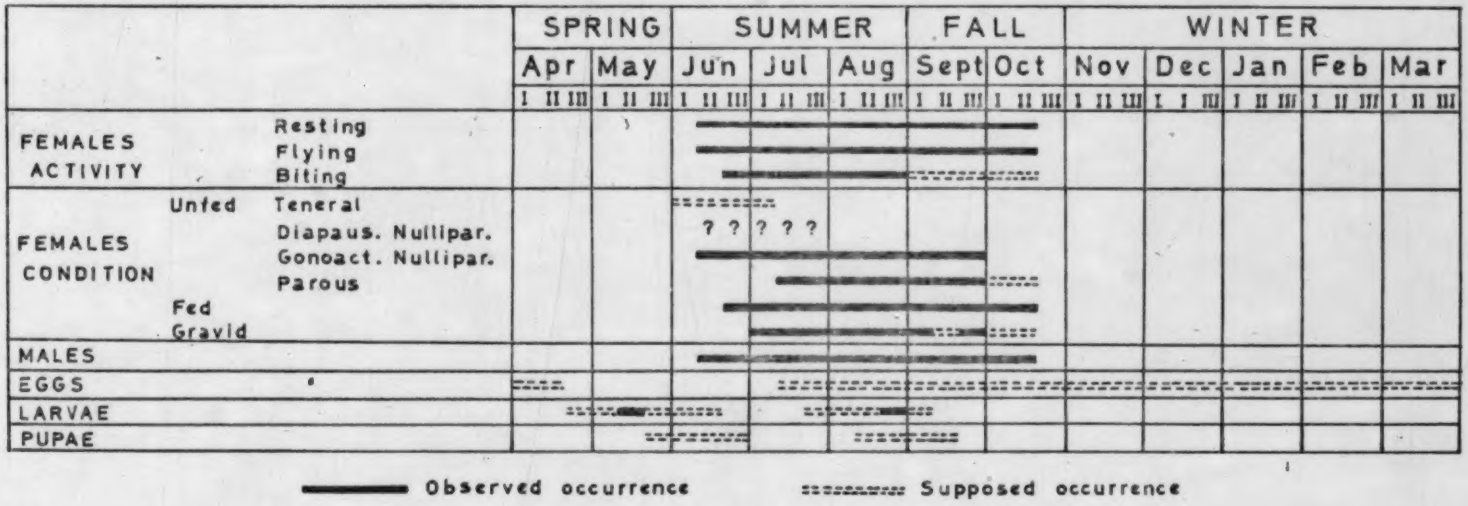


Fig.50. Summary of seasonal biology of *Cs. m. dyari*.

3.14. *Culiseta silvestris minnesotae*

Only 50 adults were taken, most of the males in September and October and 5 of the 8 females in May, (Table 23). Two females were taken in late May, 1972 in modified Malaise traps (Hocking, 1970), as part of another project. The female taken in the Malaise trap on 10/v/72 was nulliparous. A gravid was taken on a window on 7/vii/76. It had 117 eggs, rounded at both ends like those of *Cs. inornata* and unlike those of *Cs. m. dyari*. All the other females collected were unfed; none were dissected.

Forty-seven larvae were collected from the lakeside pond, (Table 24). One of the adults reared from the collection of 30/viii/73 was a *Cs. m. dyari*, but all other adults bred out in 1973 and all IV instar larvae examined from 1974 were *Cs. s. minnesotae*. It cannot be excluded, however, that some of the other early instar larvae were not *Cs. m. dyari*. The early appearance of females in spring suggests that they overwinter and this was confirmed by finding one in a snow-covered rockpile in mid-March, (see Chapter 7). Graham (1968) took females during May, July and August in Malaise traps close to the lake shore. It is odd that only one specimen was found in the box shelters, some of which were close to the site of Graham's Malaise traps and to a known breeding site. The collection of larvae from June to September suggests more than one generation per year.

Some additional adults may have been misidentified as *Cs. inornata* (females only) and *Cs. m. dyari* (males and females), particularly in the early stages of the project.

Table 23. *Culiseta silvestris minnesotae* adults collected at Edmonton and George Lake, 1972-76.

		Edmonton		George Lake		Total	
		F	M*	F	M	F	M
May	I	1	0	0	0	1	0
	II	0	0	0	0	0	0
	III	2	0	2	0	4	0
June	I	0	0	0	0	0	0
	II	0	0	0	0	0	0
	III	0	0	0	0	0	0
July	I	1(g)	0	0	0	1	0
	II	0	0	0	6	0	6
	III	0	0	0	0	0	0
Aug	I	0	0	0	1	0	1
	II	1	0	0	1	1	1
	III	0	0	1	0	1	0
Sept	I	0	2	0	6	0	8
	II	0	5	0	0	0	5
	III	0	0	0	14	0	14
Oct	I	0	5	0	1	0	6
	II	0	0	0	0	0	0
	III	0	0	0	1	0	1
Total		5	12	3	30	8	42

(g) gravid. All others unfed.

*F = Female, M = Male.

Table 24. *Culiseta s. minnesotae* larvae and pupae collected from the lakeside pond, 1973-75.

	Actual numbers collected					Total	Estimated no. per 100 dips (a)
	I	II	III	IV	Pupae		
1973							
August II	0	0	0	3	0	3	3
III	0	1	1	7	10	19 (b)	12
September I	0	0	0	1	3	4	3
II	0	0	0	1	5	6	3
III	0	0	0	0	1	1	1
1974							
June III	0	0	1	0	0	1	1
July I	0	0	5	4	0	9	9
II	0	0	1	2	0	3	2
1975							
June II	0	0	1	0	0	1	1
TOTAL	0	1	9	18	19	47 (b)	

(a) To nearest whole number.

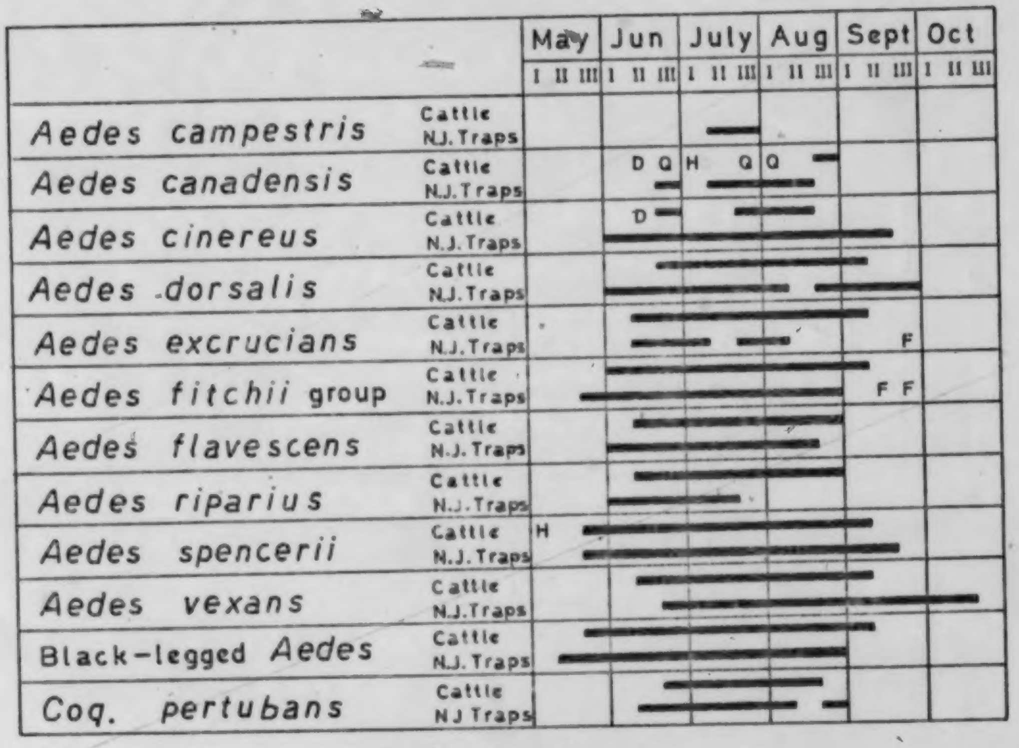
(b) One *Cs. m. dyari* reared from the sample of 30/viii/73

3.15. Notes on *Aedes* and *Coquillettidia*

Seasonal distributions of each of the species in New Jersey traps and at cattle are shown in Fig. 51, and seasonal abundance of five of the more common *Aedes* species according to the two methods are shown in Fig. 52 and 53. The points in Fig. 51 are the mean logs (n + 1), but the points in Fig. 52 are arithmetic means converted to logarithms for ease of plotting.

Over 80 % of the mosquitoes collected in the New Jersey traps were *Aedes vexans* females, but 37,147 of the 61,383 females (60.5 %) in the trap at George Lake were taken on two nights (12,245 on 15/vii/73 and 24,902 on 13/viii/75, the latter estimated from a subsample). *Ae. flavescens*, *Ae. spencerii* and the *Ae. fitchii* group were more abundant at cattle than they were in the New Jersey traps. *Ae. spencerii* was taken on humans as early as 11/v in 1972, and on 22/v/74 there were many on cattle at George Lake, though I collected few. *Ae vexans*, *Ae. spencerii* and *Ae. dorsalis* were a nuisance at George Lake until mid or late September in 1973 and 1975.

The New Jersey trap data suggest that *Ae fitchii* and *Ae. flavescens* had only one generation per year, and *Ae. spencerii* had two or three generations or broods. The numbers of *Ae. dorsalis* reached a peak in July in all 3 years, but there was a June peak only in 1974 and a September peak only in 1973. *Ae. vexans* numbers reached their main peak in mid-July, and smaller peaks in late August and late September may represent second and third generations or broods. A few were taken in mid-October, 1975, though this species does not



SUPPLEMENTARY RECORDS

D = Dry ice baited traps

Q = Quail baited traps

H = On Humans

F = On Flowers

Fig. 51. Seasonal distribution of *Aedes* and *Coquillettidia* females captured at cattle and in New Jersey traps.

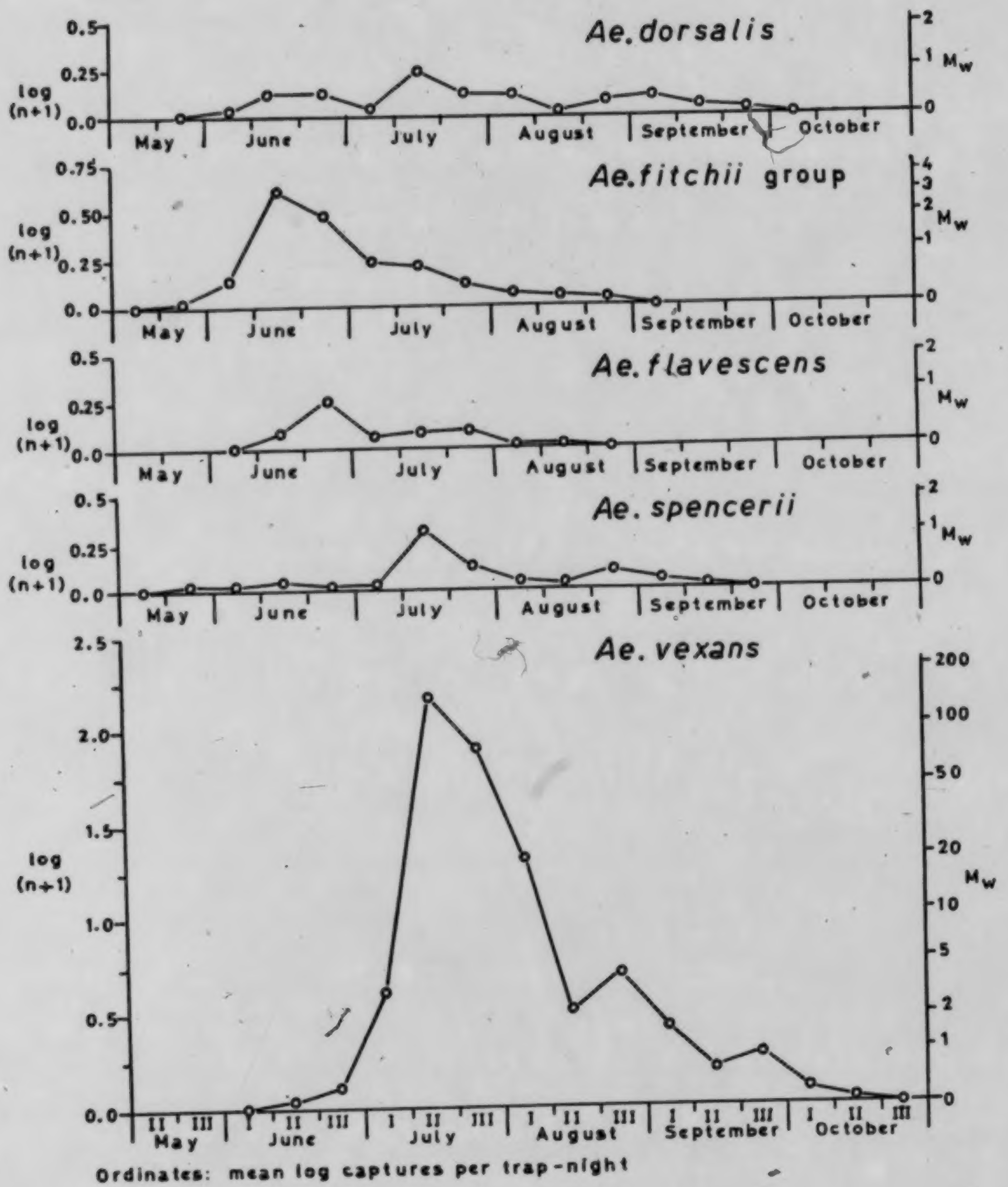
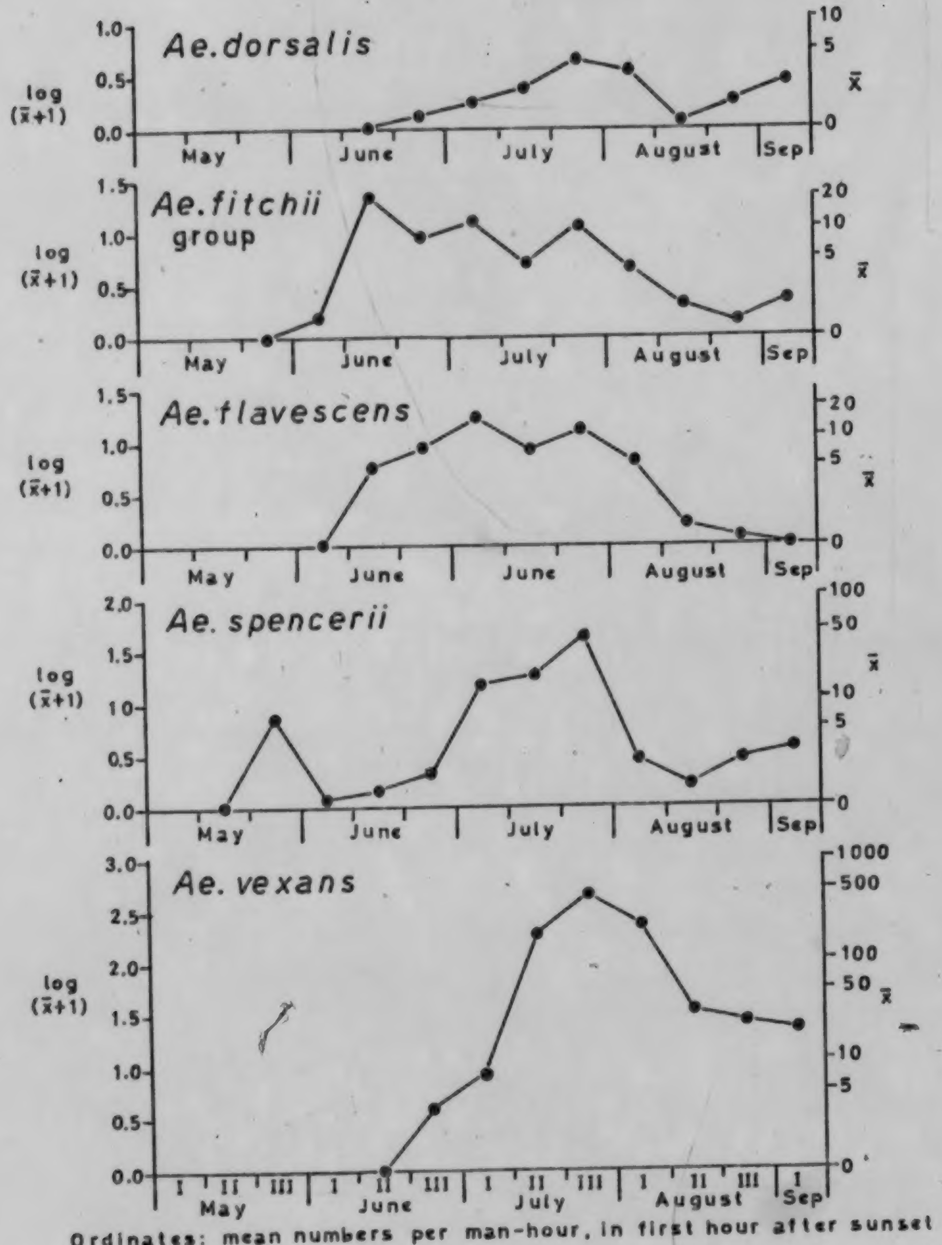


Fig. 52. Seasonal abundance of *Aedes dorsalis*, *Ae. fitchii* group, *Ae. flavescens*, *Ae. spencerii* and *Ae. vexans* females in New Jersey traps, 1973-75. M_w = Williams' Mean.



Ordinates: mean numbers per man-hour, in first hour after sunset

Fig. 53. Seasonal abundance of *Aedes dorsalis*, *Ae. fitchii* group, *Ae. flavescens*, *Ae. spencerii* and *Ae. vexans* at cattle, George Lake, 1973-75.

tolerate cold, (see Chapter 8).

The term "*Aedes fitchii* group" covers those females that keyed to *Ae. fitchii* using Carpenter and LaCasse (1955). Enfield (1976) reported two species in the Calgary area, *Ae. mercurator* Dyar and *Ae. barri* Rueger (= *euedes* Howard, Dyar and Knab), which are not included in Carpenter and LaCasse (1955), and the adult females are similar to those of *Ae. fitchii*. Enfield has also found specimens of *Ae. barri* and *Ae. mercurator* in collections from Edmonton, some previously identified as *Ae. stimulans*. It is now doubtful whether any of the Alberta records for *Ae. stimulans* are valid. I have therefore used the term "*Ae. fitchii* group" instead of the "*Aedes stimulans* group" of Barr (1958), but the choice of "type species" is arbitrary. It is clear that more fundamental work is needed on this group of species, which are severe pests in the Edmonton river valley during June.

My "black-legged *Aedes*" group is the "black-legged *Ochlerotatus* group" of Graham (1969b) and others and the "*Aedes communis* group" of Barr (1958). I have not learned to separate the adult females to species, and many of the specimens were unidentifiable because they were rubbed. Graham (1969b) recorded 11 species of this group from George Lake: *Ae. cataphylla, communis, diantaeus, hexodontus, implicatus, intrudens, pionips, pullatus, punctor, sticticus* and *trichurus*. I sent 70 females collected during a trial of repellents in 1973 to the Biosystematics Research Institute, Agriculture Canada, Ottawa. Their staff confirmed all Graham's records except *Ae. hexodontus* and *trichurus*. They also found two specimens of *Aedes abserratus* (Felt and Young), one from George Lake on 7/vi/73 and one from 24 km east of Athabasca on 17/vii/73. This is a new record for Alberta.

Some flower-visiting records for *Aedes* are given in Tables 5 and 6.

3.16. Discussion

Overwintered, nulliparous *An. earlei*, *Clx. territans*, and *Cs. alaskaensis* were seen within two weeks of snowmelt, but *Cs. inornata* did not appear until about 7 weeks after snowmelt, and the first collected each year were almost all pars and gravids. The same phenomenon was noted in Colorado at around 40° N by Dow, LaMotte and Crane (1976), in resting collections under bridges. The first *Clx. tarsalis*, which appeared in February and March, were nullipars or bloodfeds but the first *Cs. inornata*, which appeared in late March, were all gravids and pars and the first nullipars appeared in early May, after larvae had been seen. The following explanations for my results may be considered:

a) Overwintering by gravids and pars. This seems very unlikely because few gravids or pars were taken in September and none in October, though many females were examined.

b) Overwintering and first blood meal outside the study areas, and subsequent immigration by gravids and pars. This would explain the time lag, and the blood meal identifications suggest the blood-feds move at least 2.4 km in summer. The preponderance of gravids on the windows in summer, far from any known feeding site suggest that gravids too may disperse far. *Oscinella frit* and *Drosophila funebris* disperse actively between ovipositions, (Johnson, 1969). Long flights would deplete the females' energy reserves, however, and there would be few nectar sources at this time of year to replenish them.

c) Feeding by females on a host at the overwintering site, leaving the site when gravid. This would also explain the time lag and could be of great epidemiological importance if the host was a reservoir of Western Encephalitis, such as Richardson's Ground Squirrel. There is no evidence for this in Shemanchuk's (1965) study of overwintering in burrows, however.

d) Feeding after leaving the overwintering site on an unusual host such as a bird. Some support for this view comes from the finding by Dr. R. Brust (in litt.) of 10 blood-fed *Cs. inornata* in late May in Winnipeg, 9 of which had fed on passerine birds and 1 on a bird and a horse. The quail-baited traps were put out from early May with this idea in mind but *Cs. inornata* were never taken, even in summer. If feeding had taken place in the study areas it is surprising that no feds were found since digestion would have been slow at this time of year.

e) Feeding on cattle, the main hosts, soon after snowmelt but at some time in the diel when the cattle were not examined. I consider this the most likely explanation, since the cattle were not examined throughout any 24-hour cycle and a brief spell of warm weather would have been enough for most of the population to feed. It is nonetheless odd that no stragglers were taken in any of the catches at cattle, and no blood feds were taken at other sites.

f) Autogenous production of the first egg batch. Harwood (1966) has shown that the expression of autogeny in *Clx. tarsalis* is affected by daylength. If *Cs. inornata* females mature eggs autogenously on emerging from diapause, it would put an additional strain on their reserves, already depleted during winter. It is hard to see what advantage they would gain from neglecting plenty of available hosts in the area, except time, and the first larvae were found after those of the other species.

Graham (1969b) caught a number of *Cs. inornata* in the Malaise trap at George Lake in the first half of May, 1966, but he does not give a parity rate until early June, when most of the few examined were parous.

The F:G ratio seems to be a good indicator of the onset of diapause in the population. Females with "diapausing" follicles were not found at bait, and their mass appearance at other sites coincided with the cessation of blood feeding. The upper limit of 1.5 used by Spielman and Wong (1973a) for the F:G ratio of diapausing *Clx. pipiens*, seems to be applicable to *Clx. territans* and *Cs. inornata* also, but for *An. earlei* and *Cs. alaskaensis* an upper limit of 2.0 is more appropriate. In all species there were a few females with F:G ratios above these upper limits during the fall. In *An. earlei*, *Cs. alaskaensis* and *Clx. territans* some of the pars had F:G ratios below the upper limit set for the diapausing females, thus the F:G ratios are not a substitute for examining for parity.

It was impossible using the F:G ratio to distinguish between females that were truly in diapause and gonoactive post-tenerals whose follicles were still growing. Further study may reveal other age-related changes to distinguish these groups. Presence of meconium and lack of insemination were not reliable indicators of the teneral state. In the meantime, the best indicator of recent post-tenerals in a sample is the presence of tenerals.

The "primary resting stage" of follicle development in anautogenous nulliparous individuals is the stage at which development is arrested until a blood meal is taken. This stage varies between individuals of a species and between species (Rosay, 1969). In Anophelines the primary resting stage usually seems to be stage II. Mer (1936) showed that females of *An. sacharovi* Favr. (= *elutus* Edw.) with follicles in stage I would not produce eggs after a single blood meal in the laboratory, and he considered the restriction of follicle development to stage I in females in the fall to be the factor producing "semi-hibernation" (gonotrophic dissociation). Variation in the follicle stage of nulliparous females in my bait catches may represent individual variation in the primary resting stage, or may indicate that some females seek their first blood meal before the primary resting stage is reached. Both factors may be involved. All *An. earlei* females at bait had follicles in stage IIa or IIb, most *Cs. alaskaensis* and *Cs. inornata* had follicles in stage IIa, and most *Cs. m. dyari* had follicles in stage I. Most nulliparous *Cs. inornata* and all *Cs. m. dyari* taken at bait would have emerged the same season, while all *Cs. alaskaensis* and most *An. earlei* would have emerged the previous season, thus in the last two species the advance of the follicles to the resting stage was post-diapause development.

The "secondary resting stage" is the stage of arrest of follicles of parous females before their second blood meal. Several authors (e.g. Christophers, 1911, p. 78; Detinova, 1962, p. 39; Bertram, 1962, p. 201) state that the secondary resting stage cannot be earlier than stage II, because the secondary follicles have already reached this stage when the first batch of eggs is laid. Rosay (1969) showed this generalisation did not apply to 4 culicine species, in which the secondary resting stage was usually stage I, but she considered the earlier generalisation was true for two anopheline species. Most of the parous *An. earlei* that I took at bait had follicles in stage II, most *Cs. inornata* and *Cs. alaskaensis* had them in stage I, and the numbers of *Cs. m. dyari* in stages I, IIa and IIb were almost equal. Thus the follicles of some or most of the females had not reached stage II by the time the first eggs were laid, but this does not identify the secondary resting stage, and it may not be the same in all individuals. The most important conclusion here is that the follicle stage alone does not indicate whether a female is gonoactive or diapausing.

Fatbody ratings were poor indicators of diapause. Some pars of all species had fatbody ratings of 3. All *Cs. alaskaensis* and *Cs. inornata*, in September seemed to be in diapause, but less than half had fatbody ratings of 3. It was generally true however, that fatbody development in the population was greatest after diapause had set in. Spielman and Wong (1973a) noted that in *Culex pipiens* in Boston the first "fat" females (equivalent to my rating of 3) appeared in mid-September, about 2 weeks after they entered diapause and they interpreted diapause as "a transient condition that precedes hypertrophy of the fatbody". The value of the early appearance of diapause in local species may be that it permits uninterrupted nectar feeding before the flowers are over and so ensures an adequate winter food reserve. The seasonal availability of nectar and other carbohydrate sources must be an important factor in determining the optimum time of onset of diapause. Late appearance of diapause will increase reproductive potential but may decrease winter survival.

4. EXPERIMENTS WITH WILD-CAUGHT, DIAPAUSING FEMALES

4.1. Introduction

Field data for *Anopheles earlei*, *Culex territans*, *Culiseta alaskaensis* and *Culiseta inornata* suggest that they do not take blood or produce eggs in fall (Chapter 3) or winter (Chapter 7). Follicle growth, blood-feeding and assimilation in wild-caught, diapausing females was studied under laboratory conditions to determine the duration and intensity of diapause.

4.2. Materials and methods

Culiseta and *Culex* females were collected from windows on the University of Alberta campus and *Anopheles* females from box shelters at George Lake and the root cellar at Ellerslie (see Chapter 7). Some *Cs. inornata* females in 1973 were held in a cubical cage of side 35 cm, in a room at 2 - 3 C and 12 hr daylength, and given 10 % sucrose on cotton wool pads. The mosquitoes in the other experiments were held in groups of up to 20 in plastic cups or paper-lined plastic pill vials covered with netting, or in the plastic tubes supplied with the WHO kit for testing the susceptibility of adult mosquitoes to insecticides. A soaked raisin was placed on each container, and the containers were kept in incubators or in the culture room, (methods in Section 2.14.). Most females were stored in the dark at 2 C for some days between collection and the beginning of the experiments.

For blood-feeding exposures *Anopheles* and *Culiseta* females were transferred to a netting-covered 250 ml beaker held against my forearm for 15 minutes, in the light at 20 - 23 C. A western toad (*Bufo boreus*), from George Lake on 19/viii/75 sat unrestrained on moist sand in a 3.6 litre glass jar in the culture room, and *Culex territans* females were introduced through a small hole in the netting cover. The first exposure lasted 16 hr overnight and later exposures 6 hr during the photophase. Blood-fed mosquitoes were dissected 5 - 8 (usually 7) days after feeding.

Culiseta inornata midguts for protein and trypsin assays were dissected out and stored with a few drops of 0.7 % NaCl in screw cap vials in a freezer. Each midgut was homogenized in 1 ml tris buffer (pH 7.9), centrifuged at 14,500 g for 10 minutes, and 5 subsamples of 0.1 ml withdrawn from the supernatant fluid, 2 for protein and 3 for trypsin assays. For protein assays each subsample was reacted with alkaline copper and folin phenol and the absorbance read at 750 nm, (method of Lowry et al, 1951). The absorbance readings were converted to μg/ml (= μg/female) by comparison with a standard curve prepared using bovine serum albumin. For trypsin assays, two of the subsamples were incubated with 1.0 ml of 2.0 mM BApNA in 50 mM tris buffer (pH 7.9) for 15 minutes at 30 C, then 0.5 ml of 30 % acetic acid was added to stop the reaction. For a reagent blank, BApNA was incubated alone and the third midgut subsample added after the acetic acid. The absorbance was read at 410 nm, and the reagent blank reading subtracted from each assay reading, (method of Huang, 1971). The absorbance for both assays was read on a Beckman DU-2 spectrophotometer.

4.3. Results

4.3.1. *Anopheles earlei*

A female *An. earlei* was considered in diapause if she had no follicles in stage II and an F:G ratio no greater than 2.0, (Chapter 3). All females collected from box shelters at George Lake from 18 - 19/ix/74 and dissected immediately were in diapause, (Table 25a). The rest were offered blood, but only 2 of 20 fed at the first exposure and none at later exposures. Both feds were still in diapause after 7 days holding in an outdoor cupboard, at a mean (natural) daylength of 12:50 hours and a daily mean temperature of 15.8 C. Those that refused blood were held in the lab at 16 hours/ 20 C and dissected 7 days after the first exposure. The majority had emerged from diapause and the mean F:G ratio was 2.32.

From September ~~74 to~~ March 75, monthly samples of *An. earlei* were removed from the root cellar where the mean temperature was 6 C, (see Chapter 7). By 18/i/75, only 33 % of those dissected immediately had non-diapause follicles (Table 25b), but 12 of 20 took blood at the first exposure to my arm, and another 5 fed in 6 more daily exposures, making a total of 17 fed (85 %). Blood-feds and unfeds were held at 16 hours/20 C. Eight of 16 feds laid eggs on moist filter paper and there was some hatch from five of the ovipositions. Five more died without laying but dissection revealed they had mature eggs, (Table 25c). Thus 81 % of the blood-feds matured eggs, more than would have been expected from the 33 % of unfed females with non-diapause follicles. The mean F:G ratio was

Table 25. Ovarian development of *Anopheles earlei* in the laboratory and in a root cellar.

	Number examined	% in stage I	IIa	IIb	Mean F:G ratio	% in diapause
(a) Collected from box shelters, George Lake, 18-19/ix/74.						
Dissected immediately	12	100	0	0	1.72	100
Blood-fed, held 7 days at 12:50 hr/15.8 C	2	100	0	0	1.80	100
Unfed, held 7 days at 16 hr/20 C	15	33	40	27	2.32	4
(b) Collected from root cellar, dissected immediately.						
Date						
17/ix/74	12	100	0	0	1.67	100
16/x/74	10	100	0	0	1.91	100
15/xi/74	16	100	0	0	2.02	88
16/xii/74	15	100	0	0	2.08	67
18/i/75	12	67	22	22	2.20	67
14/ii/75	11	100	0	0	2.27	36
14/iii/75	6	67	22	22	2.30	67

(c) Collected from root cellar on 18/i/75, fed 1-7 days later. Held at 16 hr/20 C. Dissected 7 days after feeding.

Number examined	% in stage I	IIa	IIb	V	% laid eggs
16	0	7	12	31	50

higher for the February than for the January females, but no yolk was seen in the follicles. Two of 6 mosquitoes collected in March had follicles with yolk. The fatbody of these mosquitoes appeared to be nearly exhausted, and starvation may have caused inhibited yolk deposition. Of 67 *An. earlei* collected from rockpiles during February and March 1975, 10 (15 %) all from the same pile had follicles in stage II, (see Chapter 7).

4.3.2. *Culex territans*

Females collected from 18 - 22/ix/74 were stored at 2 C until 17/x/74. Ten of 12 (83 %) dissected on removal from storage, were in diapause, but only 4 of 6 (67 %) held 7 days and 4 of 11 (36 %) held 14 days, were in diapause (Table 26). The mean F:G ratio was only 1.73 for the 3 females in stage IIa and 1.90 for the 3 females in stage IIb, both lower than average values for this species (see Section 3.2.).

Females collected from 2 - 10/ix/75 were first exposed to the toad on 15/ix/75 but none fed. After 3 exposures 1 week apart, 1 of 15 held at 16 hr/20 C fed, and produced eggs 1 week later, while 2 of 12 held at 12 hr/20 C fed but did not produce eggs, (Table 27a). Of the females not exposed to the toad, all those dissected immediately were in diapause. All those held at 12 hr/20 C for up to 21 days remained in diapause, but of those held at 16 hr/20 C, 33 % had emerged from diapause after 14 days and 25 % after 21 days, (Table 27b).

Table 26. Ovarian development in diapausing *Culex territans* before and after holding at 16 hr/20 C.

Holding period	Number examined	—% in stage— I	IIa	IIb	Mean F:G ratio	% in diapause
None	12	100	0	0	1.38	83
7 days	6	100	0	0	1.53	67
14 days	11	44	28	28	1.64	36

Table 27. Blood feeding and ovarian development in diapausing *Culex territans* before and after holding at 16 hr/20 C or 12 hr/20 C.

(a) Feeding

Holding conditions	Day of 1st exp.	Day of last exp.	No. of exposures	Total no. exposed	No. fed	% fed	No. of feds maturing eggs
No holding	1	-	1	10	0	0	-
12 hr/20 C	7	21	3	12	2	17	0
16 hr/20 C	7	21	3	15	1	7	1

(b) Ovarian development of unfeds

Holding regime	Duration of holding	No. examined	% in stage I	% in stage II	Mean F:G ratio	% in diapause
No holding		20	100	0	1.28	95
12 hr/20 C	7 days	6	100	0	1.27	100
	14 days	4	100	0	1.27	100
	21 days	4	100	0	1.21	100
16 hr/20 C	7 days	6	100	0	1.20	100
	14 days	3	100	0	1.30	67
	21 days	4	100	0	1.30	75

4.3.3. *Culiseta alaskaensis*

Females collected from 2 - 8/vii/73 fed readily, 39 of 56 (70 %) taking blood at the first exposure. None of the blood-feds was dissected, but there was no external sign of egg maturation, and there was copious spotting of apparently undigested blood on the inside of the holding tube.

The aim of the next experiment was to see if a fairly brief exposure to a temperature below 0 C would arouse females from diapause. Females collected between 13/vii/75 and 1/viii/75 were transfered on 5/viii/75 from storage in the dark at 2 C to 16 hr/20 C, and supplied with raisins. This raisin feeding period was introduced because the mosquitoes appeared very thin when captured, and meconium was shed on the bottom of the cage, indicating that some were newly emerged. The mortality during this period was 39 % (79 of 204). On 10/viii/75 the survivors were divided into 4 groups: 1) dissected immediately, 2) offered blood, 3) maintained at 16 hr/20 C on raisins, and 4) transferred to the dark at -5 C for 7 days then returned to 16 hr/20 C for another 7 days, when some were offered blood and others dissected. Of the females dissected immediately, 88% were in diapause with stage N2 follicles, (Table 28). A few unfed females emerged from diapause after holding at 16 hr/20 C, with or without the exposure to -5 C. Most of the females were alive at the end of their exposure to -5 C, but 19 of 66 (29 %) had died within 7 days of their return to 16 hr/20 C. Of the females offered blood, 32 % fed at the beginning of the experiment, but only 5 % fed after 7 days at -5 C and 7 days at 20 C. None of the blood-feds matured eggs.

Table 28. Blood feeding and ovarian development in diapausing *Culiseta alaskaensis* before and after 7 days exposure to -5 C.

Blood-feeding rates: before chilling 32% (7 of 22), after chilling 5% (1 of 20). Feds dissected 7 days after feeding, at 16 hr/20 C.

Holding (1)	Number examined	Number insem-inated	% in stage N2	I	I-II	IIa	IIb	Mean F:G ratio	% in diapause
Unfeds									
None	16	6	88	12	0	0	0	1.28	88
7 days	7	1	1	4	0	1	1	1.78	43
14 days	7	1	0	5	2	0	0	1.45	71
7 days -5 C + 7 days	12	0	0	11	1	0	0	1.47	58
7 days -5 C + 14 days	11	0	0	8	1	1	1	1.59	54
Feds									
None	7	6	0	5	0	2	0	1.68	43
7 days -5 C + 7 days	1	0	1	0	0	0	0	1.60	100
Total	61	14							

% inseminated = 23.0

(1) See text for details.

Only 23 % of the females were inseminated, though males were abundant at the site of collection.

4.3.4. *Culiseta inornata*

When females collected on 25/ix/73 were offered blood the same day, 72 % of 29 fed. The feds were divided into two groups, one held at 8 hr/10 C and the other at 16 hr/20 C, but none matured eggs, (Table 29). Several hundred more females collected during September 1973 were held at 12 hr/2 C, for 3 months. Mortality was high and the 49 females offered blood on 31/xii/73 were most of the survivors. Only 5 fed (10 %) but all of them matured eggs after 7 days at 16 hr/ 20 C.

All females collected from 13 - 22/ix/74 were in diapause but 73 % fed when offered blood on the day of transfer from 2 C to 20 C (Table 30). None of the blood-feds matured eggs but 92 % had emerged from diapause by 7 days after feeding and within this group 1 female (8 %) had follicles in stage III. All unfeds had emerged from diapause after 7 days at 16 hr/20 C. The blood-feeding rate was the same as before, but 71 % of the blood-feds matured eggs.

The aim of the next experiment was to see if increases in both daylength and temperature were necessary to arouse females from diapause, or an increase in temperature would suffice. (Since the females had been stored at 2 C, the effect of increase in daylength alone could not be investigated.) Females collected 11 - 28/ix/75 were stored at 2 C until 29/ix/75. All those dissected on removal from storage were in diapause, and 91 % were still in diapause after

Table 29. Blood-feeding and ovarian development in diapausing *Culiseta inornata* before and after 3 months at 12 hr/2 C.

Holding before feeding	No. exposed	% fed	Holding after feeding	Number dissected	% with mature eggs
None (coll. 25/ix)	29	72	5 days at 8 hr/10 C	10	0
			5 days at 16 hr/20 C	6	0
3 months at 12 hr/2 C	49	10	7 days at 16 hr/20 C	5	100

Table 30. Blood-feeding and ovarian development in diapausing *Culiseta inornata* before and after holding at 16 hr/20 C. (1974).

Duration of Holding	Number exposed	% fed	Number dissected	% in stage I	II	III	V	Mean F:G ratio	% in diapause
Not offered blood									
No holding	-	-	10	100	0	0	0	1.28	100
7 days	-	-	15	80	20	0	0	1.78	0
14 days	-	-	23	26	74	0	0	2.36	0
Offered blood									
No holding before, 7 days after	37	73	12	67	25	8	0	1.90[(a)]	8
7 days before, 7 days after	48	73	33	3	23	3	71	2.36[(a)]	0

(a) Mean F:G ratio is for the females which did not mature eggs beyond stage II.

14 days at 16 hr/20 C and 100 % still in diapause after 14 days at 12 hr/20 C, (Table 31). Of those offered blood on removal from storage, 48 % fed and 8 % of the feds matured eggs. Only 40 % of those held at 16 hr/20 C and 37 % of those held at 12 hr/20 C for 7 days took blood, and 20 % of the blood-feds in each group matured eggs.

Digestion of blood was studied in diapausing females, captured 2 - 10/ix/75, stored at 2 C and fed on 15/ix, and also in non-diapausing females, laboratory-reared at 16 hr/20 C. The non-diapausing females were given raisins until 2 - 6 days after emergence, and only those which took blood at the first exposure, when they were 5 - 9 days old, were used in the experiment. Fed females were held at 16 hr/20 C and at intervals after feeding, groups of 5 were lightly anaesthetised with ethyl acetate, and dissected. The quantity of blood in the midgut was scored as 3 = distended, 2 = filled, 1 = trace of blood, and 0 = no blood. The remaining females were transferred to a clean holding tube and at the next interval the degree of spotting of blood on the inside of the tube scored as 2 = many spots, 1 = few, 0 = none. Ten diapausing and 10 non-diapausing females from the same sources as those used in the experiments had mean wing lengths of 6.3 and 5.0 mm and mean unfed weights of 5.4 and 3.0 mg, respectively.

After exposure to my arm most females in both groups had enough blood in their midguts to be visible without dissection. Those without visible blood were dissected immediately, and a small amount of blood found in a further 16 % of the diapausing and 2 % of the

Table 31. Blood feeding and ovarian development in diapausing *Culiseta inornata* before and after holding at 16 hr/20 C or 12 hr/20 C. (1975).

All feds were dissected 7 days after feeding.

Duration of holding	Daylength	Number exposed	% fed	Number dissected	% in stage N2	I	IIa	IIb	V	Mean F:G ratio	% in diapause
Not offered blood											
No holding	-	-	-	22	5	95	0	0	0	1.29	100
7 days	16 hr	-	-	10	0	100	0	0	0	1.22	100
7 days	12 hr	-	-	10	0	100	0	0	0	1.24	90
14 days	16 hr	-	-	11	0	100	0	0	0	1.37	91
14 days	12 hr	-	-	10	0	100	0	0	0	1.22	100
Offered blood											
No holding	-	25	48	12	0	75	8	8	8	1.76	64
7 days	16 hr	25	40	10	0	70	10	10	10	-	-
7 days	12 hr	27	37	10	0	90	0	0	10	-	-

non-diapausing females, (Table 32). Five females from each group with no blood visible on dissection were the "unfeds", thus they were self-selected. In both blood-fed and unfed diapausing females only stage I follicles were seen and the mean F:G ratio never exceeded 1.5 (Fig. 54). In the unfed non-diapausing females the mean F:G ratio was 2.0 and after feeding it rose steadily until by 96 hours it was 13.0, and 3 of the 5 females examined had mature eggs. The midgut was distended with blood in 4 of 5 diapausing females less than one hour after feeding, but by 24 hours after feeding 4 of 5 midguts contained only a trace of blood. Blood was present in some of the hindguts and on the inside of the holding tubes at all time intervals up to 76 hours. In the non-diapausing females the midguts of all individuals remained distended with blood until at least 48 hours after feeding. There was a little blood in one of the hindguts and in the holding tube 12 hours after feeding, but no more blood was seen in any of the hindguts until 72 hours after feeding, when a black residue of digested blood appeared in 4 hindguts. In the diapausing females the blood darkened but was always distinctly red. It was dark in some non-diapausing individuals, 12 hours after feeding and black in all of them by 48 hours after feeding.

At less than 1 hour after feeding the protein content of the midguts of the diapausing females was only half that in the non-diapausing females (Fig. 54) in spite of the smaller size of the latter. To compensate for the difference in meal size, the protein contents from 12 hours onwards were converted to percentages of the values at less than 1 hour after feeding. This reveals, (Fig. 55), that loss of protein from the midgut was more rapid in the diapausing females. The copious

Table 32. Observations on blood feeding and ovarian development in diapausing (wild-caught) and non-diapausing (laboratory reared) *Culiseta inornata*.

(a) Feeding.

	Number exposed		% of	
		obvious feds	cryptic feds	unfeds
Diapausing	45	64	16	20
Non-diapausing	53	72	2	26

(b) Digestion and ovarian development (n = 5).

Time after meal (hr)	(x) Distension of midgut				Blood in hind-gut	(y) spots in tube	Colour of blood in midguts	Follicle Stage						
	3	2	1	0				N2	I	IIa	IIb	III	IV	V
Diapausing														
Unfed	0	0	0	0	0	-	-	0	5	0	0	0	0	0
1	4	1	0	0	1	-	Fresh Red	1	4	0	0	0	0	0
12	3	2	0	0	5	2	Fresh Red	2	3	0	0	0	0	0
24	0	1	4	0	3	2	Fresh or Dark Red	0	5	0	0	0	0	0
36	1	2	1	1	2	2	Dark Red	0	5	0	0	0	0	0
48	1	0	3	1	2	2	Fresh or Dark Red	0	5	0	0	0	0	0
76(n=2)	0	0	1	1	1	2	-	0	2	0	0	0	0	0
Non-diapausing														
Unfed	0	0	0	0	0	-	-	0	4	1	0	0	0	0
1	5	0	0	0	0	-	Fresh Red	0	4	1	0	0	0	0
12	5	0	0	0	1	1	Fresh or Dark Red	0	0	1	4	0	0	0
24	5	0	0	0	0	0	Red-Black	0	1	0	3	1	0	0
36	5	0	0	0	0	0	Red-Black	0	0	0	1	4	0	0
48	5	0	0	0	0	0	Black	0	0	0	0	5	0	0
72	4	1	0	0	4	0	Black	0	0	0	0	0	5	0
96	0	2	0	3	2	0	Black-Empty	0	0	0	0	0	2	3

(x) Distension of midgut: 3 = Distended, 2 = Filled, 1 = Trace, 0 = Empty.

(y) Spotting of blood on sides and bottom of tube: 2 = Many spots, 1 = Few, 0 = None.

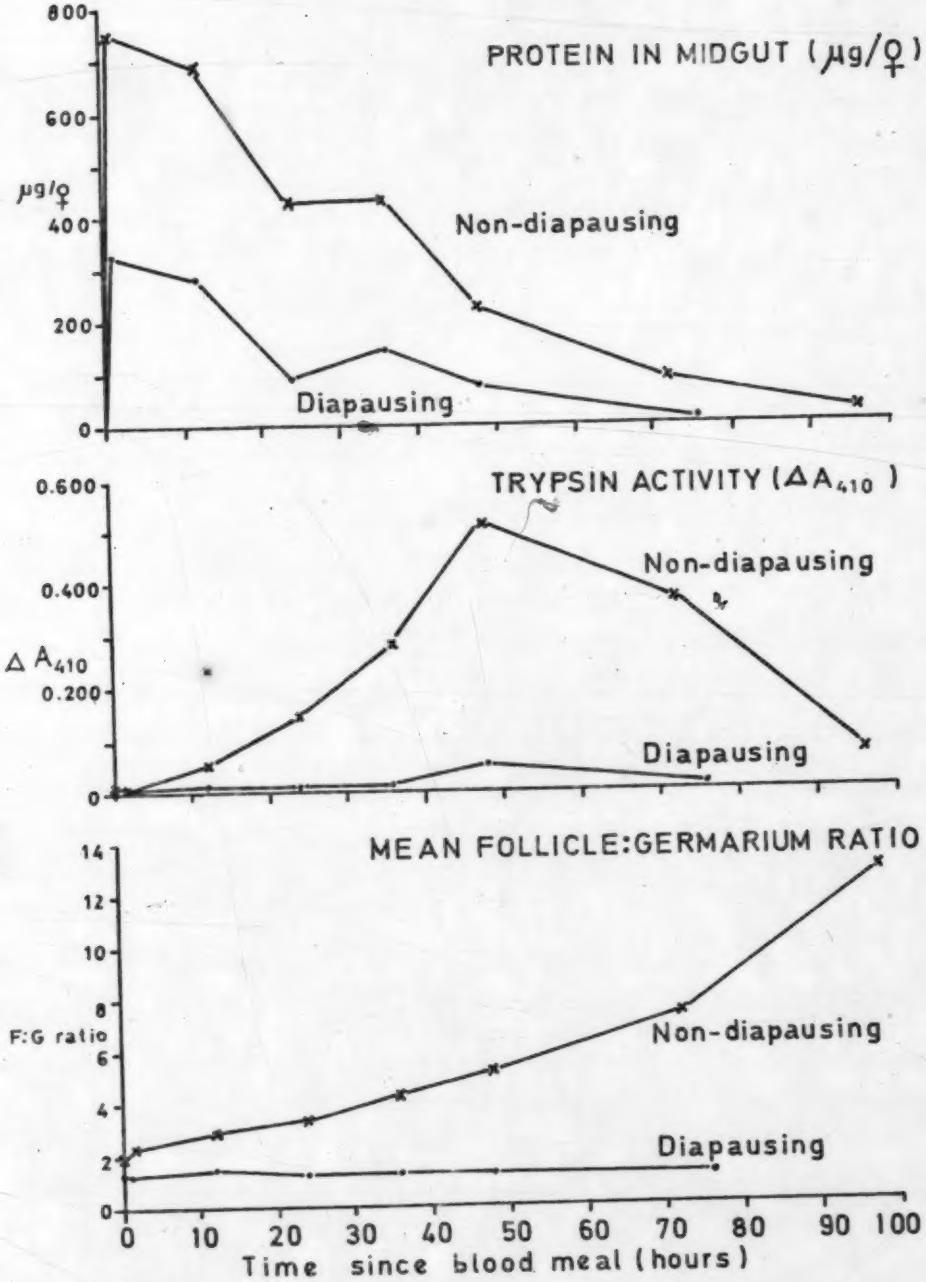


Fig. 54. Protein in midgut, trypsin activity and F:G ratios in blood-fed, diapausing and non-diapausing *Cs. inornata*.

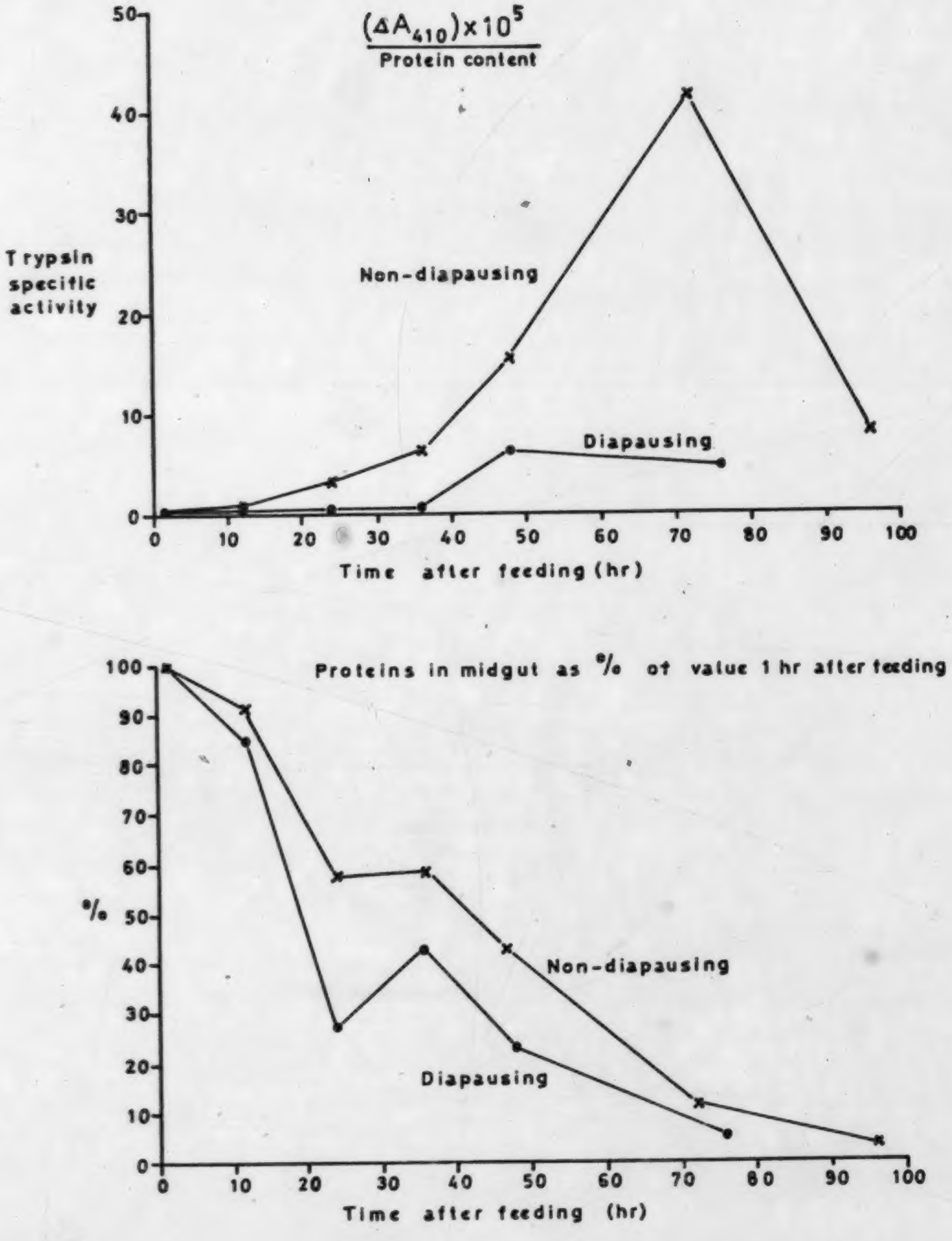


Fig. 55. Specific trypsin activity and relative protein content of midgut in blood-fed, diapausing and non-diapausing *Cs. inornata*.

shedding of blood, the very low trypsin activity in all but one of the diapausing females and the failure of eggs to develop suggest that the more rapid loss of protein was not the result of more rapid digestion. One female 48 hours after feeding, had a protein content of 301 μg and a ΔA_{410} value of 0.218; the protein content is within but the ΔA_{410} below the range for the non-diapausing females at this time. The trypsin activity of the non-diapausing females was first noted 12 hours after feeding, reached a peak at 48 hours after feeding, and had greatly decreased again by 96 hours after feeding. When the data are converted to specific activity, by dividing the mean ΔA_{410} value by the mean protein content, the peak of activity is more pronounced and shifts to 72 hours after feeding, (Fig. 55).

4.4. Discussion

It is hard to draw any conclusions from these experiments because the few that were repeated gave inconsistent results. Three possible sources of error were the small numbers of females used, variations in the duration and conditions of storage before use, and the unknown age of the females at the time of collection.

Some, but not all samples of *Cs. inornata* and *Cs. alaskaensis* showed high blood feeding rates without any previous holding in the laboratory, and the readiness to take blood could not be predicted from the rate of ovarian diapause at the time of feeding. Very few *Culex territans* fed on a toad after 3 weeks in the laboratory, and the ovaries remained in diapause, but I have no evidence that even non-diapausing females would feed on such a host under these conditions. *Anopheles earlei* could not be induced to feed in September, but fed readily and produced eggs immediately after removal from the root cellar in January. The few *Cs. inornata* that survived and fed after 3 months at 2 C all produced eggs. Since a period of chilling terminates diapause in some other insects, such as grasshopper eggs (reviewed by Lees, 1955), the period of storage at 2 C which preceded most of the experiments may have been more important than any of the holding regimes that followed. However, females that were aroused from diapause and those that were not were both exposed to 2 C beforehand. An exposure to -5 C did not arouse *Cs. alaskaensis* from diapause, and the *An. earlei* in the root cellar completed diapause development without exposure to temperatures below 0 C, unless they experienced an early frost before going in.

In those cases where blood feeding was followed by egg development there was always some sign that the ovaries had emerged from diapause at the time the mosquitoes had fed. This raises the question of whether the ovary itself could exert some influence on digestion and assimilation of blood. The ovary is known to produce ecdysone, which influences the production of the female specific protein, vitellogenin, in the fatbody, (Hagedorn, 1974). Alternatively, the midgut and the ovary could both be under the control of the neurosecretory system. This question will be considered in Chapter 9.

I do not consider the results obtained with *Cs. alaskaensis* and *Cs. inornata* to be sufficient reason to describe these species as displaying gonotrophic dissociation, firstly because this phenomenon was never seen in nature, and secondly because the ingested blood was not shown to be used to increase the food reserves of the females and did not even appear to be digested. De Buck, Schoute and Swellengrebel (1932) were able to force feed a few *Anopheles maculipennis messeae* and although no eggs were produced, they do not call this "gonotrophic dissociation", since the blood did not appear to be digested. Instead of being passed out, as in my *Culiseta*, the blood separated into two distinct layers, visible externally, and remained for a long time in the gut, often killing the female.

5. DEVELOPMENT, TEMPERATURE AND THE PHENOLOGY OF DIAPAUSE

5.1. Introduction

In Chapter 3 the dates of onset of reproductive diapause in the mosquito populations were estimated from the cessation of attacks on bait animals, the disappearance of blood-feds and gravids, and increases in the numbers of females with small follicles. In this chapter the time of onset of diapause is related to daylengths and temperatures in nature. Since diapause in the adult is affected by conditions experienced by the aquatic stages, (see Chapter 6), an attempt has been made to reconstruct the life histories of *Cs. inornata* females back to the dates of hatching. This has been done by estimating the number of day-degrees required for the female life cycle, and the number of day-degrees in nature, based on air and water temperatures recorded at George Lake.

5.2. The durations of the larval, pupal and teneral stages and of the gonotrophic cycle in *Cs. inornata* at 10, 15 and 20 C.

The durations of 4 stages of the life cycle were measured at 3 constant temperatures in the laboratory. The larval stage was treated as the median time in days from egg hatch to the larval-pupal ecdysis. The duration of the pupal stage was estimated by subtracting the larval stage from the median time from egg hatch to eclosion (pupal-adult ecdysis). The teneral stage was treated as the median time, in hours, from eclosion to the disappearance of larval muscle remnants from the abdomen. Beklemishev (1940, as described by Detinova, 1962) divided the gonotrophic cycle into

3 phases: (1) the search for a host and the attack, (2) the digestion of the blood meal and maturation of the ovaries, and (3) the search for a suitable body of water and the oviposition. Only the duration of the second phase was measured in *Cs. inornata*; this phase is probably the longest and the most closely related to temperature. The first and third phases are more under the influence of diurnal rhythms, and behavioural responses to natural conditions much harder to reproduce in the laboratory.

5.2.1. Materials and methods

Mosquitoes for all 4 experiments were taken from the Edmonton I and II colonies, which were maintained at 16 hr/20 C, (section 2.14.). The mosquitoes were kept in incubators accurate to ± 1 C. In the measurement of the duration of the larval and pupal stages, half the mosquitoes at 20 C and all those at 10 C were kept under daylengths of 8 hr. Mosquitoes in all the other experiments were kept under 16 hr daylengths.

The durations of larval and pupal development at 10 and 20 C were measured as part of Diapause Induction Experiment 1, (see Section 6.1.). The durations of these stages at 15 C were measured 2 years later with larvae from the Edmonton II colony, but the methods used were the same.

To measure the duration of the teneral stage, pupae from the Edmonton II colony were examined at hourly intervals, and newly emerged females transferred to the incubators where they were kept in netting-covered plastic cups in plywood boxes, lit by two 0.15 A incandescent bulbs. Raisins were supplied from emergence onwards, and an equal number of males was added to each group of females. Groups of females were dissected at intervals of 6 hours (20 and 15 C) or 12 hours (10 C). The timing of the dissections was arranged to give at least four groups of ten females, some with and some without muscle remnants.

In the experiment to determine the duration of blood digestion, 8 - 10 day old females from the Edmonton II colony were fed on my arm. Raisins were supplied until 1 - 3 days before the blood meal. Fed females were kept singly in plastic vials, covered with netting, in dessicators over saturated NaCl solution, which gave a relative humidity of 75 ± 3 % (Peterson, 1947). The dessicators were kept in incubators lit by cool white fluorescent tubes. The females were dissected on the day when remnants of the blood meal were no longer visible in the midgut by external examination with a hand lens. Those females that had not produced eggs were excluded from the results. Any females that matured eggs before emptying the midgut would have been missed, but the latter condition was never seen in wild-caught females. The results shown are the combined results of four experiments. In the first experiment, the females were given no food after the blood meal and mortality at 10 C was high. In the other experiments, each female had a water-soaked cotton ball continuously and a soaked raisin for one day in every three. Mortality was much lower with this extra food. The median durations of the larval and pupal stages were estimated by plotting cumulative percentage pupation and eclosion on logarithmic probability paper. The median duration of the teneral stage was estimated by plotting, on arithmetic probability paper, the percentages of individuals with muscle remnants. The results for the durations of blood digestion are expressed as arithmetic means.

Expected durations of the 4 stages at each temperature were calculated using the hyperbolic and Krogh-Jorgensen equations. The hyperbolic equation, (or temperature-summation formula) may be written:

$$y = \frac{k}{t - t_o}$$

or in the reciprocal, straight-line version:

$$v = a + bt$$

where y = the duration of the process, t = temperature, t_o = the developmental zero or temperature threshhold, v = velocity of development = 1/y, and a, b and k are constants. It may be shown that $t_o = -a/b$. This equation has been used by many workers, including Shlenova (1938, as described by Detinova, 1962) who made a detailed study of the effects of temperature and humidity on the rate of blood digestion in *Anopheles maculipennis*. The constants a and b were calculated by the method of least squares. Nielsen and Evans (1960) showed that the relation between temperature and the velocity of pupal development was described better by the Krogh-Jorgensen equation than by the hyperbolic or several other equations. The Krogh-Jorgensen equation may be written:

$$v = a + bc^t$$

where c is a new constant and the other notation is the same as for the hyperbolic equation. When c = 1.00, the values of a and b will be identical for the two equations. The values of a, log b and log c were calculated using the methods given by Nielsen and Evans (1960). As an index of the agreement between observed and calculated values, the root mean square deviation between them was expressed as a percentage of the observed value at 15 C.

5.2.2. Results and discussion

Pupation and eclosion of each sex at each temperature was nearly synchronous, except that at 10 C a few individuals were much slower than the rest, (Fig. 56). Mortality at 10 and 15 C was over 50 %, (Table 33), most deaths occurring in the early instars, but the adult sex ratio was close to 1.0. The males developed more rapidly than the females at all temperatures (Table 33). The expected durations of the larval stage in females, according to the hyperbolic equation, were close to my observed values (Fig. 57), and slightly higher than the values reported by Hanec and Brust (1967) and Rosay (1973). This may simply have been because their values are for both sexes combined and mine are for females only. Shelton's values (1973) are lower than anyone else's, possibly because he used the minimum rather than the median or mean times of development, and his results are inconsistent, the larvae apparently developing faster at 12 than at 15 C.

The median duration of the teneral stage was 124.0, 58.0 and 33.5 hours at 10, 15 and 20 C, respectively, (Table 34). The meconium was lost long before the muscle remnants, and a median time cannot be estimated from the data. Less than one hour after emergence at 20 C, the ovarioles in 7 of 10 females examined had

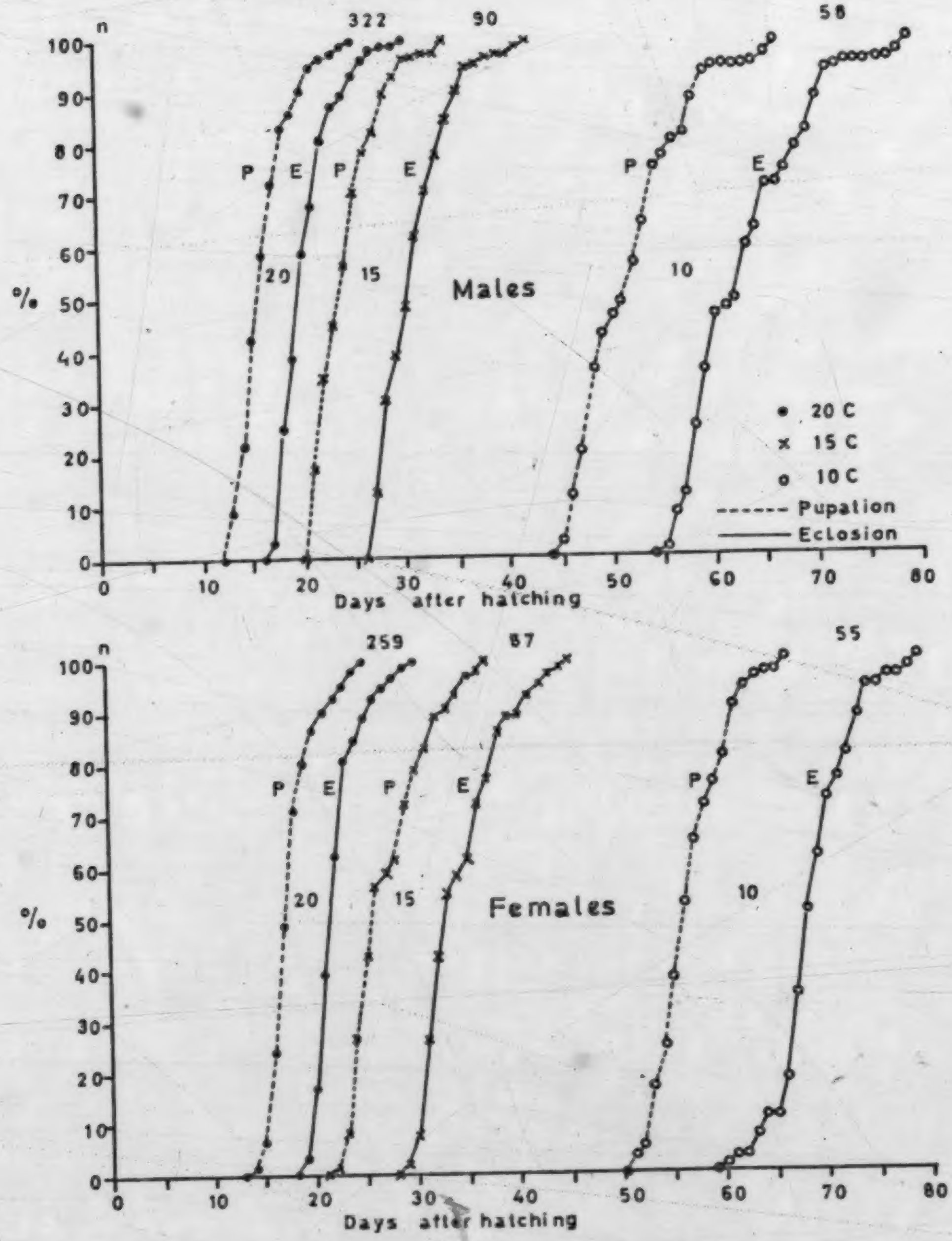


Fig. 56. Cumulative pupation and eclosion of *Cs. inornata* males and females at 10, 15 and 20 C.

Table 33. Mortalities and median development times of *Culiseta inornata* larvae and pupae at 10, 15 and 20 C.

	MORTALITIES		
	10 C	15 C	20 C
Number of			
I instars	400	400	800
Pupae	121	214	653
Adults	116	177	582
Mortality (%)			
Larvae	69.8	46.5	18.4
Pupae	1.3	9.4	8.9
Total	71.1	55.9	27.3

	MEDIAN TIMES (days)					
	10 C		15 C		20 C	
	M	F	M	F	M	F
Hatching to eclosion (a)	61.4	68.2	29.9	33.0	19.5	21.4
Hatching to pupation (b)	50.4	55.8	23.2	26.1	15.8	17.0
Pupal stage (a-b)	11.0	12.4	6.7	6.9	3.7	4.4

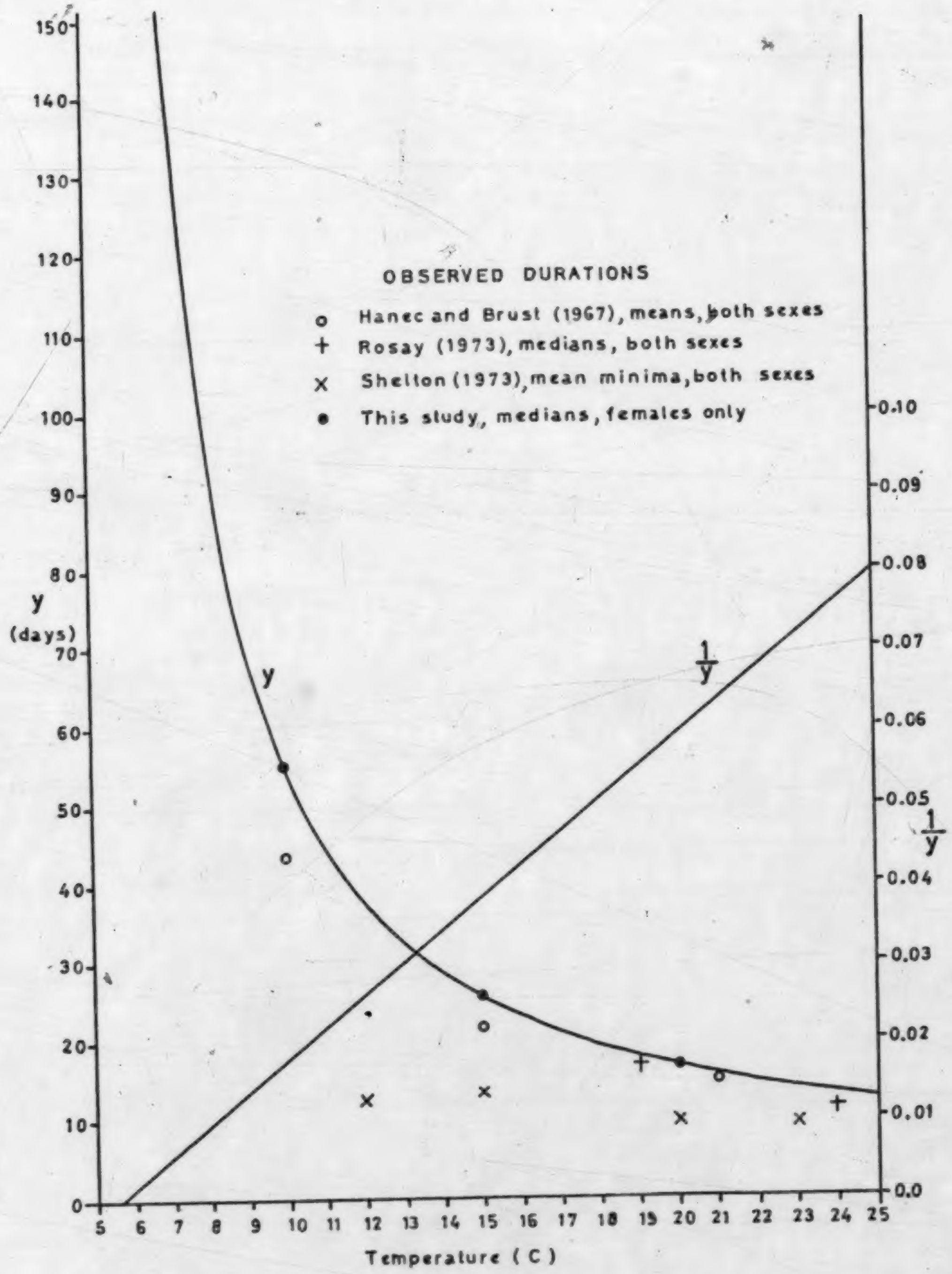


Fig. 57. Observed and calculated durations and rates of larval development of *Cs. inornata* females at temperatures of 5-25 C.

Table 34. Observations on teneral *Culiseta inornata* females at 10, 15, and 20 C.

20 C. n = 10 at each observation time. Median time to loss of muscle remnants=33.5 hr.

Hrs after emergence	<1	24	27	30	33	36	42	48
Number with muscle remnants	10	10	10	7	5	2	2	0
Number with meconium	10	3	3	0	0	0	0	0
Mean F:G ratio	1.25(2)	1.28	1.23	1.28	1.35	1.24	1.39	1.37
Number "in diapause"(1)	10	10	10	10	8	10	8	9
Number in stage N1	7	0	2	0	0	0	0	0
N2	3	6	4	7	2	3	0	2
I	0	4	4	3	8	7	10	8

15 C. Median time to loss of muscle remnants = 58.0 hr.

Hrs after emergence	36	42	48	54	60	66	72
n	5	6	10	10	10	10	10
Number with muscle remnants	5	6	9	7	3	2	0
Number with meconium	3	1	2	1	0	0	0
Mean F:G ratio	1.23	1.28	1.25	1.23	1.28	1.28	1.52
Number "in diapause"	5	6	10	10	10	10	5
Number in stage N1	0	1	1	0	0	0	0
N2	2	4	3	2	0	1	1
I	3	1	6	8	10	9	9

10 C. n = 10 at each observation time. Median time to loss of muscle remnants=124.0 hr.

Hrs after emergence	72	84	96	108	120	132	144	156	168
Number with muscle remnants	10	10	9	8	5	4	4	1	0
Number with meconium	1	0	0	0	0	0	0	0	0
Mean F:G ratio	1.27	1.24	1.24	1.26	1.22	1.31	1.30	1.46	1.58
Number "in diapause"	10	10	10	10	10	8	8	6	3
Number in stage N2	10	7	4	1	0	1	5	0	0
I	0	3	6	9	10	9	5	10	10

(1) "In diapause" = F:G ratio no greater than 1.5.

(2) Mean of the 3 females with follicles in stage N2.

follicles in stage N1. At the median time for loss of muscle remnants, most of the individuals at all three temperatures had an F:G ratio no greater than 1.5, hence they would have been classified as "in diapause" if encountered in field collections.

The mean time of egg maturation after blood feeding was 12.3, 7.0 and 4.8 days at 10, 15 and 20 C, respectively, (Table 35). A few of the females at 10 and 15 C showed no egg development by the time the remains of the blood meal had disappeared from the gut.

Constants for the hyperbolic and Krogh-Jorgensen equations for females in each of the four stages are shown in Table 36. The root mean square deviations between observed and expected values ranged from 0.2 % to 5.4 % for the hyperbolic equation and were all nil for the Krogh-Jorgensen equation. The developmental zeros ranged from 3.6 C for blood digestion to 6.2 C for the teneral stage, with a mean of 5.0 C. Since the value of the constant c is close to 1.00 for the larval stage and blood digestion, the temperature-velocity curves for these two stages will be almost straight lines.

For the sake of simplicity, in later sections a developmental zero of 5 C will be assumed for all stages. Andrewartha and Birch (1954) criticized the practice of obtaining developmental zeros by extrapolation, and further observations below 10 C would have been desirable. It is often difficult to get results, however, at temperatures close to the developmental zero, because of high mortalities. Hanec and Brust (1967) found that at 5 C only 15 % of their *Cs. inornata* larvae survived to pupate, after a mean of 205 days, and

Table 35. Duration of blood digestion in *Culiseta inornata* at 10, 15, and 20 C.

	10 C		15 C		20 C	
Number examined	21		26		35	
	Days after meal	No. gravid	Days after meal	No. gravid	Days after meal	No. gravid
	11	4	6	10	4	8
	12	9	7	8	5	13
	13	5	8	6	6	14
	14	3	9	2		
Mean duration of digestion (days)	12.3		7.0		4.8	
% developing follicles beyond Stage IIb	87		91		100	
Number examined	39		32		26	

Table 36. Duration of developmental processes in *Culiseta inornata* in relation to temperature, with constants for two equations.

	Hatching to pupation (days)	Pupal stage (days)	Teneral stage (hrs)	Blood digestion (days)
Observed values at				
10 C	55.8	12.4	124.0	12.3
15 C	26.1	6.9	58.0	7.0
20 C	17.0	4.4	33.5	4.8
Hyperbolic Equation				
y-intercept, a	-0.02300	-0.068993	-0.01323	-0.04638
slope, b	0.00409	0.014663	0.0021206	0.01270
Root mean square deviation(1)	0.2	4.9	5.4	0.8
Developmental zero (C)	5.6	4.7	6.2	3.6
Krogh-Jorgensen Equation				
a	-3.554983	-0.148124	-0.016470	-0.885346
b	3.532467	0.139413	0.012995	0.854369
c	1.00114	1.05077	1.06561	1.012423
Root mean square deviation(1)	0.0	0.0	0.0	0.0

(1) as % of the value at 15 C.

all the pupae died. The developmental zero for blood digestion in *An. maculipennis* varied between 4.5 and 9.9 C, depending on humidity, (Shlenova quoted in Detinova, 1962). I find a developmental zero below 5 C in *Cs. inornata* plausible, because after 10 blood fed females from the Edmonton I colony had been kept at 2 - 4 C for 30 days, the follicles had reached stage IV in 4 of them and stage III in another 3.

The calculated median time to loss of muscle remnants at 17 C in *Cs. inornata*, 45.8 hours, is lower than Rosay's values (1961) for *Culex pipiens quinquefasciatus* (= *C. p. fatigans*), 54 hours, and *Aedes nigromaculis*, 59 hours. Rosay found that expulsion of meconium from the midgut took even longer than autolysis of the muscle remnants, in both species she studied, but considered the muscle remnants to be better indicators of the teneral stage, because the time to autolysis showed less individual variation. In my own experiment expulsion of the meconium took place long before autolysis of the muscle remnants. This difference may have been due to the fact that my mosquitoes were given raisins, while Rosay's were given no food. In the experiment with teneral females the daylength at all temperatures was 16 hr, thus it is unlikely that any of the females were in diapause, (see Chapter 7), and the results confirm that it is impossible to distinguish diapausing females from recent post-teneral, gonoactive females by F:G ratio alone.

In the experiments on the duration of the teneral stage and of blood digestion, the females at 10 and 15 C did not have time to acclimate to these temperatures before the experiments began.

Another source of error lay in the fact that in three of the experiments observations were made only once per day. This may have introduced substantial errors into the estimation of the duration of the pupal stage and blood digestion at 20 C.

5.3. Estimated duration of the life cycle of *Cs. inornata* in day-degrees

The estimated duration of the life cycle in non-diapausing female *Cs. inornata* is 483 day-degrees above a threshold of 5 C, (Table 37). The degree-days required for the larval, pupal and teneral stages and for blood digestion are based on the results given in the preceding section. The requirement of 45 day-degrees for egg development (after laying) is based on the observation that egg rafts from colony *Cs. inornata* usually hatched 3 days after laying at 20 C, i.e. 3(20 - 5). The estimates of 10 day-degrees between the end of the teneral stage and the first blood meal, and another 10 between the end of blood digestion and egg laying, are based on the assumption of 1 day each at 15 C. It is likely that *Cs. inornata*, like most insects that have been studied, has a circadian rhythm of activity, and this rhythm under natural conditions may have more effect than minor temperature changes on the time of host-seeking and oviposition flights. Thus a female that completed the teneral stage before the normal time of flight activity might take a blood meal the same day, but one which completed the teneral stage later might not feed until the next day. (For a further discussion of this topic, see Gillett, 1971).

Table 37. Numbers of day-degrees above 5 C required for different stages of the life cycle of *Culiseta inornata*. Stages in parentheses not confirmed by experiment.

Stages	Number of day-degrees above 5 C
(Egg)	45
Larva	261
Pupa	66
Teneral	22
(End of teneral to first blood meal)	10
Blood digestion	69
(End of blood digestion to egg laying)	10
(TOTAL)	483

5.4. Air and water temperature records at George Lake

Air temperatures in the aspen wood and water temperatures in the Lakeside Pond were recorded as described in Section 2.13. Ten-day mean values for 3 seasons are shown in Fig. 58, and maxima, minima and means are shown in Tables 73 and 74 (Appendix). The mean water temperatures were higher than the air temperatures, except in October. Examination of the diel fluctuations in temperature (Fig. 59) shows that this was because the daily maxima were only slightly higher and the daily minima much lower in the air than in the water. The mean temperatures shown in Fig. 58, Table 73 and Table 74 are (mean daily maximum + mean daily minimum)/2. The mean temperatures calculated from the 12 days of 2-hourly readings shown in Fig. 59 are 0.8 C lower for the air temperatures and 0.2 C lower for the water temperatures than the averages of daily maxima and minima for the same days. Thus the much more lengthy process of calculating mean daily temperatures from 2-hourly readings would have brought the mean air and water temperatures 0.55 C closer together.

Haufe's study (1957) of the larval habitats of *Aedes communis* at Churchill, Manitoba showed that in an insolated pool overlying permafrost, temperature gradients of up to 16 C developed, and that the mosquito larvae formed aggregations in parts of the water close to their preferred temperature (15.5 C). The temperatures at the bottom of the Lakeside Pond were less variable and the mean maxima 1to 11 C lower than those at 2.5 cm, (Table 74). The temperature in the Lakeside Pond had a smaller differential between surface

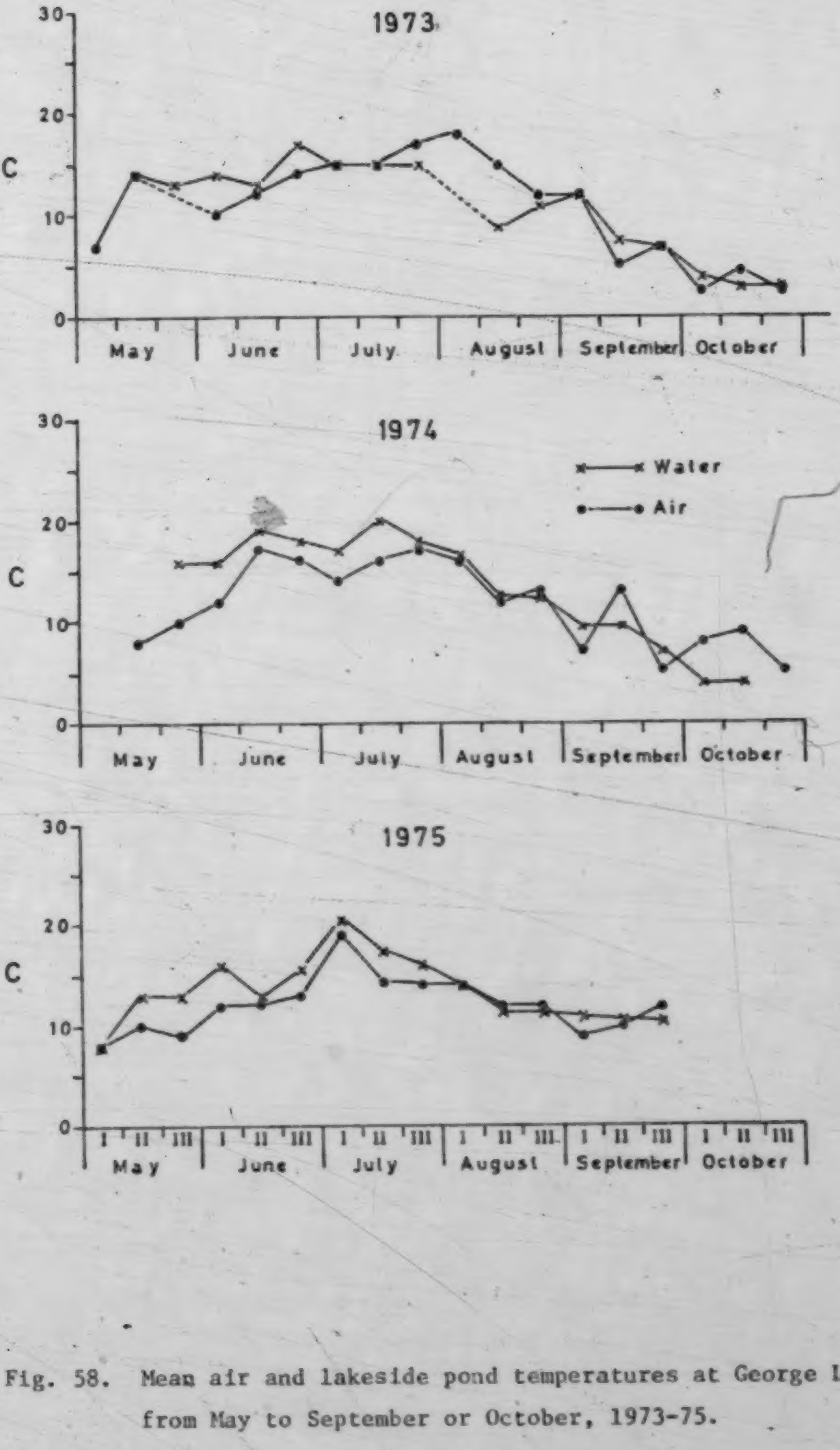


Fig. 58. Mean air and lakeside pond temperatures at George Lake, from May to September or October, 1973-75.

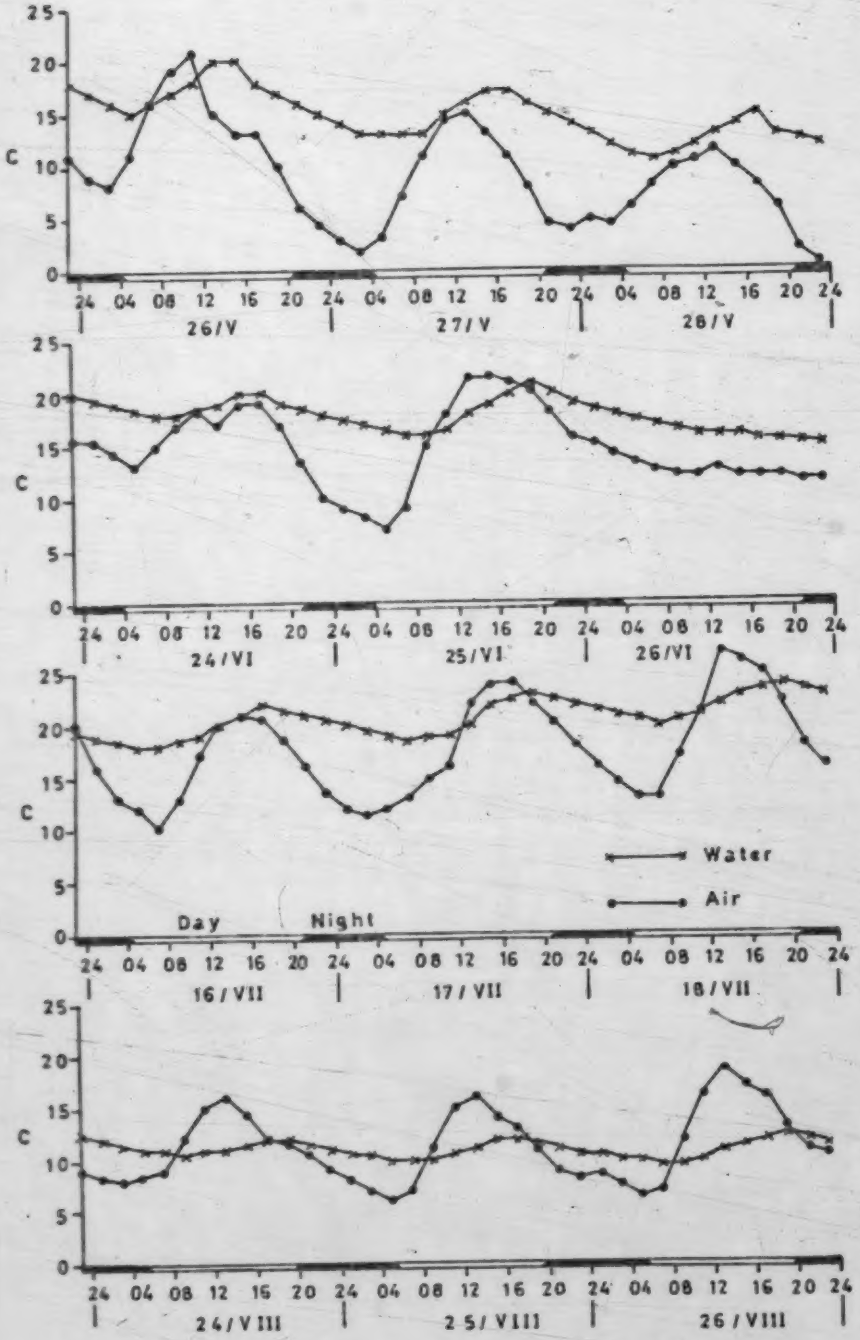


Fig. 59. Two-hourly air and lakeside pond temperatures at George Lake during 4 selected 3-day periods in 1974.

and bottom than the pool studied by Haufe (1957), probably because of the thick coating of duckweeds and the absence of a permafrost layer underlying the former. *Cs. inornata* larvae in the laboratory cultures spend most of their time at the water surface, but it was not established if they did this in the Lakeside Pond also.

It was not determined how close the Stevenson screen temperatures were to the temperatures at the mosquitoes' natural resting sites. The largest single collection of resting *Cs. inornata* at George Lake, 13 females, was in the Stevenson screen itself, but this was on 11/ix/74, after the period covered by the calculations.

5.5. Reconstruction of the life histories of the first diapausing and the last non-diapausing females

5.5.1. Methods

Using the data for 1974 and 1975 reported in Chapter 3, estimates were made of the dates when the last nullipars were taken at bait, the last gravids were taken at other sites (disregarding a few stragglers), and the date at which 50 % of the nullipars dissected were in diapause. The number of day-degrees above 5 C in air and water at George Lake in July and August, 1974 and 1975 were tabulated, (Table 75, Appendix). Air temperatures for 7 missed days were obtained from the records for Sion and water temperatures for 4 missed days estimated from the air temperatures. The life histories were then reconstructed by adding up the day-degrees, working backwards from the date of appearance of diapause, until their sum was enough to complete the various stages of the life cycle, as estimated in Section 5.3. When the date of completion of each stage of the life cycle had been estimated, the daylength (including twice Civil Twilight) was read from the curve of values for 54° N (Fig. 60), prepared from data given in List (1958). George Lake is at 53° 57' N. The temperatures at hatching and pupation are the means for the 5 days surrounding the estimated dates that these processes occurred.

5.5.2. Results

The results for *Cs. inornata* will be considered first because it was the only species for which good estimates of the duration of the

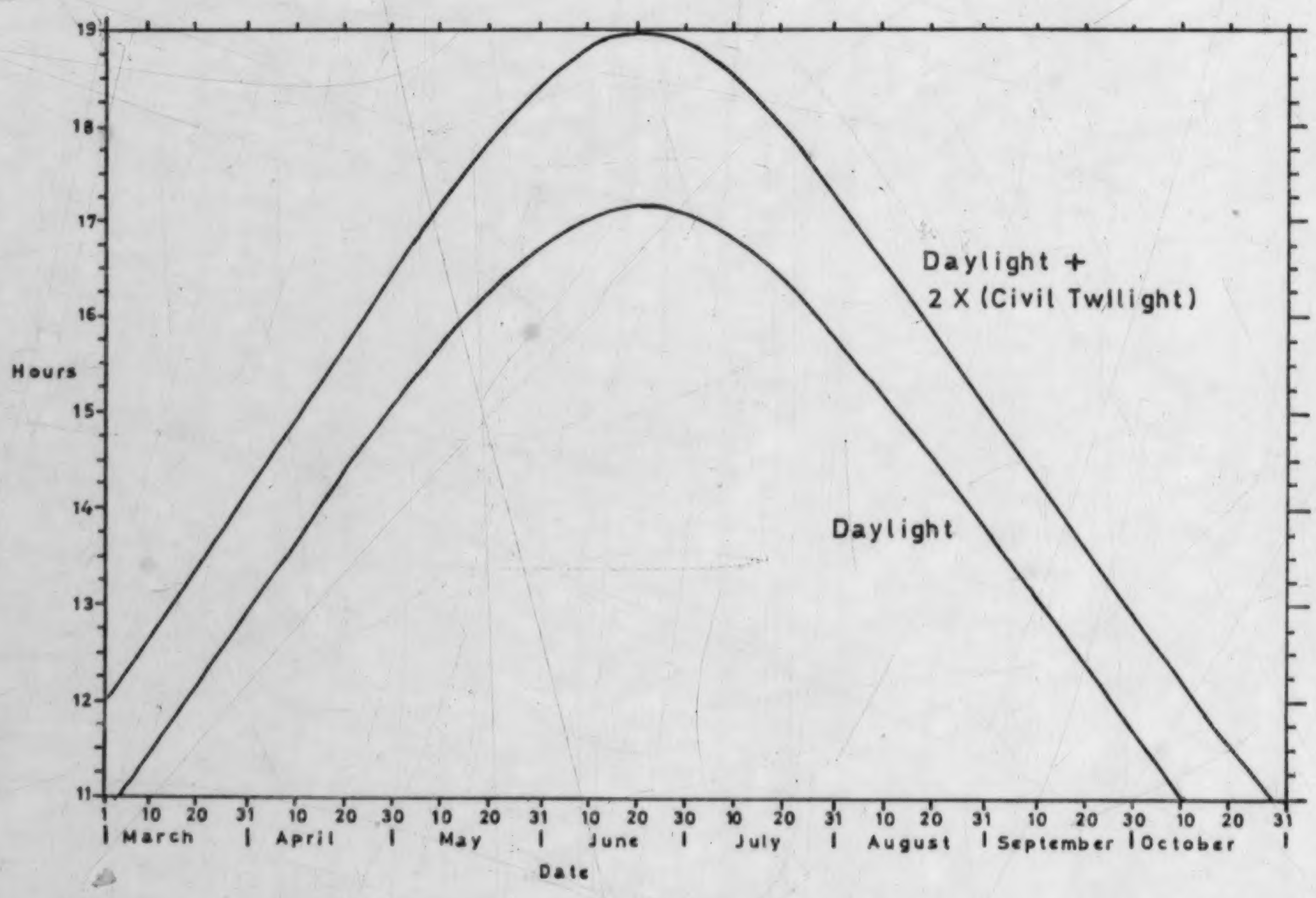


Fig. 60. Daylengths at 54^{O}N (plotted from data in List, R.J. 1958)

life cycle were available. Changes in composition of catches at bait (cattle and calf-baited trap) and other sites (mostly New Jersey traps and windows) in August 1974 and 1975 are shown in Fig. 61, and my interpretation of them is shown in Table 38. The data taken as a starting point for the reconstruction of the life histories is the middle day of a three-day period. The last nullipars were taken at bait on 20/viii/74 and 19/viii/75, but this does not prove that none bit between then and the next sampling period, 25 - 27/viii, when none were taken in 1974, and only a par and a gravid in 1975. In both years the last gravids were calculated to have fed within one day of the actual date that the last nullipars were taken at bait. The last gravids were assumed to be in their first gonotrophic cycle, since we are looking for the latest date at which gonoactive females could have been produced. One gravid female was taken in the New Jersey trap at Edmonton on 16/ix/74, but this was the only gravid female taken in mid-September of any year. Diapausing females exceeded 50 % of all the nullipars collected on 20/viii/74 and 17/viii/75.

Diapausing and gonoactive females in the two years are estimated to have hatched on 19/vii and 13/vii, and to have pupated on 9/viii and 5/viii. The last gonoactive females are estimated to have hatched as late as, or even later than, the diapausing females. Daylengths were 18:03 to 18:23 at hatching and 16:57 to 17:39 at pupation, a total decrease of 1:49 to 2:00 hours, and a rate of 3.8 to 4.1 min/day, or 0.34 to 0.38 % of the original photophase per day. The mean water temperatures were 19.0 to 20.0 C at

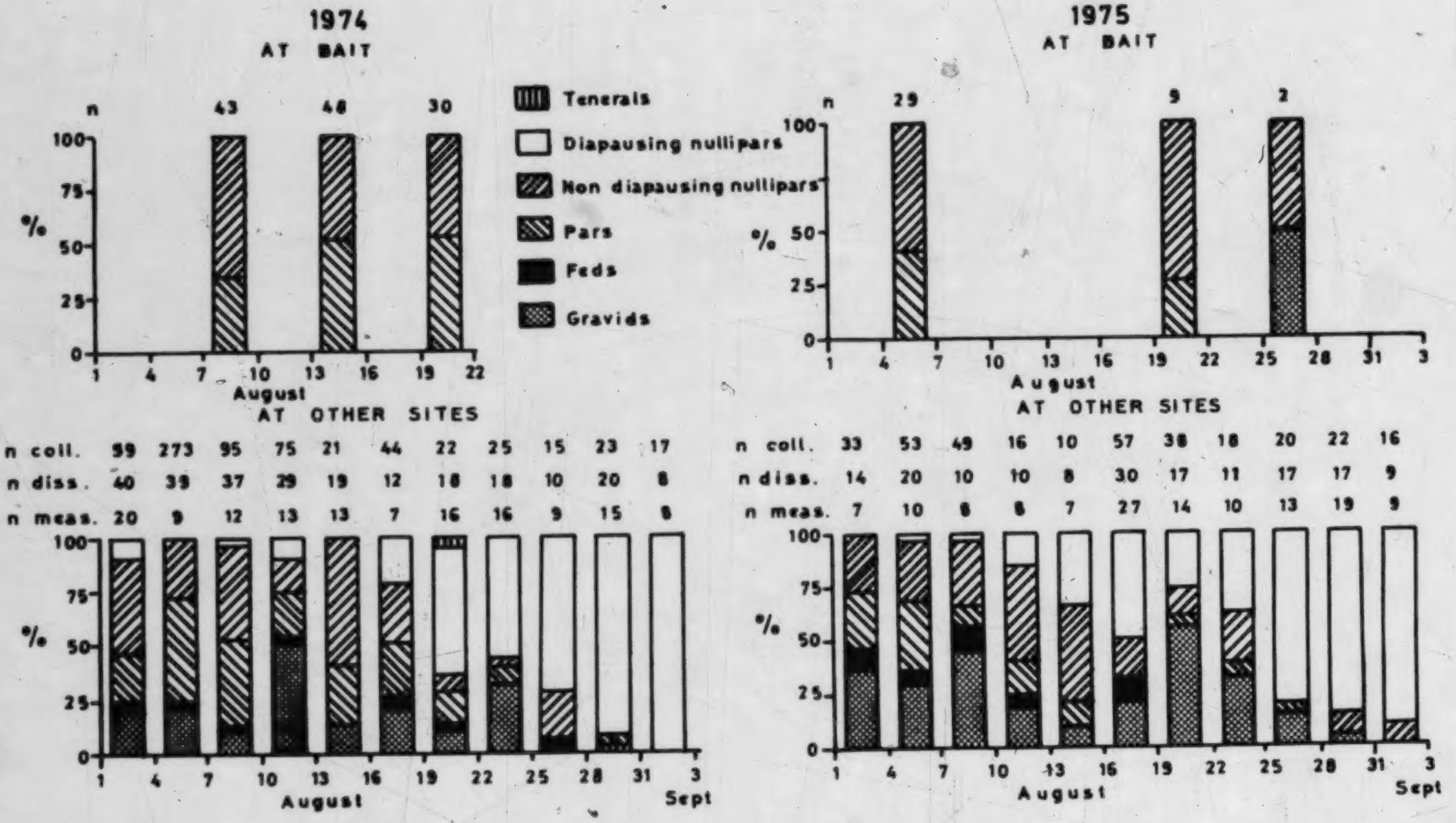


Fig. 61. Composition of catches of *Cs. inornata* for 3-day periods in August, 1974 and 1975

Table 38. Temperatures and daylengths during development of the last gonoactive and the first diapausing *Culiseta inornata*, 1974 and 1975.

	1974			1975		
	Last nipars at bait	Last gravids	50% in diapause	Last nipars at bait	Last gravids	50% in diapause
Date of collection	20/viii	30/viii	20/viii	20/viii	30/viii	17/viii
Calculated dates of:						
Hatching	19/vii	19/vii	19/vii	14/vii	14/vii	13/vii
Pupation	9/viii	9/viii	9/viii	8/viii	8/viii	5/viii
Emergence	17/viii	18/viii	17/viii	16/viii	16/viii	11/viii
Blood meal	-	21/viii	-	-	20/viii	-
Daylength (hr:min) at:						
Hatching	18:03	18:03	18:03	18:21	18:21	18:23
Pupation	17:39	17:39	17:39	16:44	16:44	16:57
Emergence	16:03	16:00	16:03	16:15	16:15	16:34
Change in daylength from hatching to emergence	-2:00	-2:03	-2:00	-2:06	-2:06	-1:49
Rate of change:						
Minutes/day	4.1	4.0	4.1	3.8	3.8	3.8
% of original daylength/day	0.38	0.37	0.38	0.34	0.34	0.34
5-day water temperature (C) around date of:						
Hatching	20.0	20.0	20.0	19.0	19.0	20.0
Pupation	14.2	14.2	14.2	13.6	13.6	14.4
Emergence	13.2	12.8	13.2	11.2	11.2	12.8
Mean temperatures of aquatic stages	16.9	16.7	16.9	15.3	15.3	16.0
Change of temperature from hatching to emergence	-6.8	-7.2	-6.8	-7.8	-7.8	-7.2
Rate of decrease, C/day	0.23	0.24	0.23	0.24	0.24	0.25

hatching and 14.2 to 14.4 at pupation, a decrease of 6.8 to 7.2 C, or 0.04 to 0.21 C/day. The decrease in temperature was not greater during the development of the diapausing females than during the development of the gonoactive females, nor was the mean daily temperature of the aquatic stages any lower.

Changes in the composition of catches of *An. earlei* in July and August, 1974 and 1975 are shown in Fig. 62 and my interpretation in Table 39. The higher proportion of feds and gravids in July and early August in 1974 is because collections of mosquitoes resting in farm buildings were made in 1974 but not in 1975. In 1974 the collections from farm buildings were continued until after the end of August but yielded only unfed, diapausing females. Most of the other specimens were collected from box shelters at George Lake. Although one fed female was taken in the Egyptian trap on 20/viii/75, the numbers at bait were always so small that I have not drawn any conclusions from them.

In both years some of the nullipars collected in early July were apparently in diapause, and in 1975 at least 50 % of the nullipars throughout July and August were in diapause. However, tenerals were frequently taken with them, which suggests that some of the post-tenerals could have been gonoactive, with follicles still growing. In 1974 approximately half the nullipars taken as late as 6 - 10/viii appeared to be gonoactive. I have chosen the median date of appearance of diapausing females as 13/viii in 1974 and 8/viii in 1975, but it could have been at least ten days earlier.

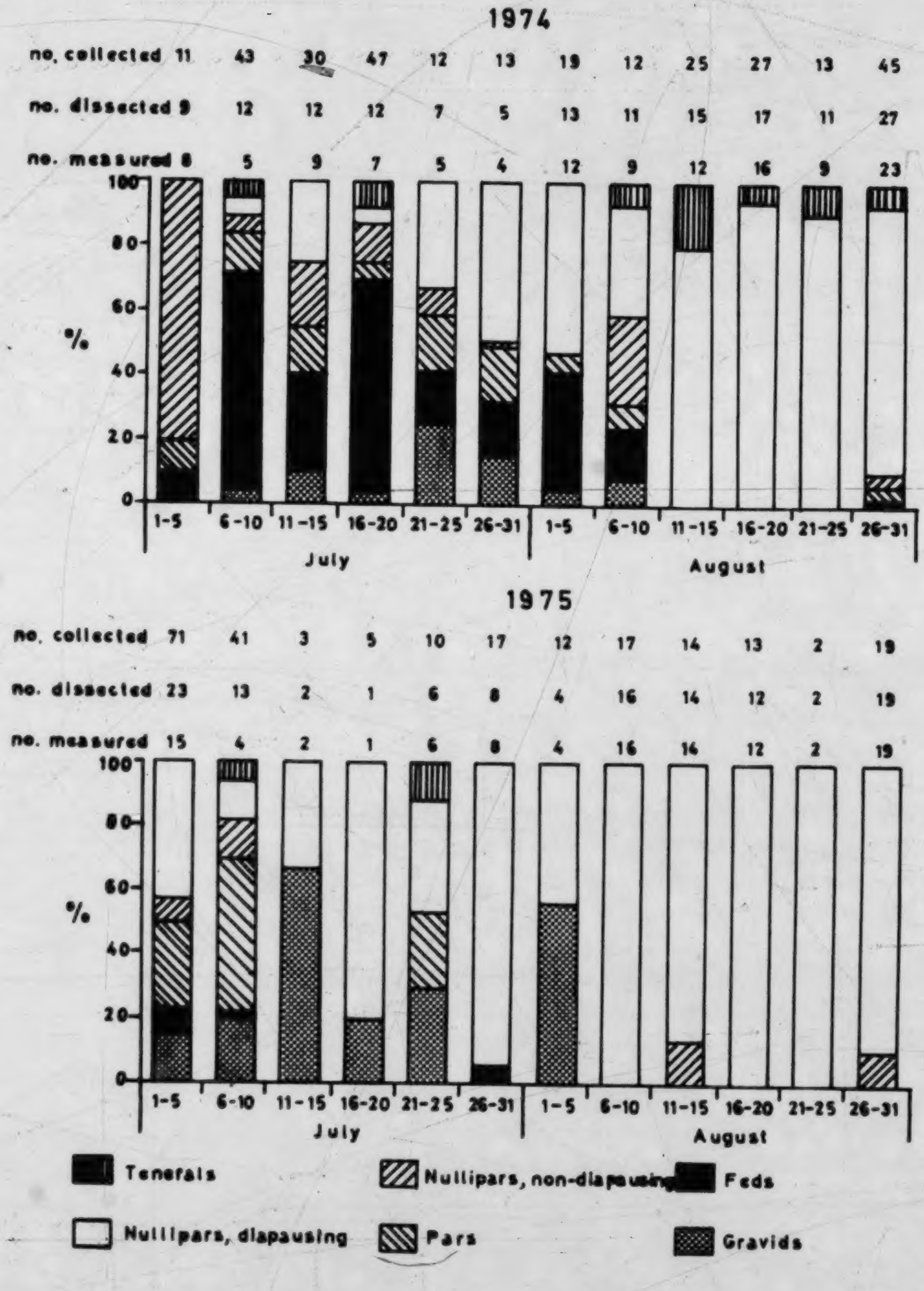


Fig. 62. Composition of catches of *An. earlei* for 5-day periods in July and August, 1974 and 1975.

Table 39. Daylengths and temperatures at hatching and pupation of diapausing *Anopheles earlei*, *Culex territans* and *Culiseta alaskaensis*, 1974 and 1975.

	Anopheles earlei		*Culex territans*	*Culiseta alaskaensis*	
	1974	1975	1975	1974	1975
Date of mass appearance of diapausing females	13/viii	8/viii	5/viii	25/vi	5/vii
Estimated date of					
Hatching	14/vii	9/vii	6/vii	26/v	5/vi
Pupation	3/viii	29/vii	26/vii	15/vi	25/vi
Daylength at					
Hatching	18:20	18:36	18:42	18:11	18:39
Pupation	17:06	17:26	17:33	18:55	18:57
Change in daylength from hatching to pupation	-1:14	-1:10	-1:09	+0:44	+0:18
Rate of change:					
min/day	-3.7	-3.5	-3.4	+2.2	+0.9
% of original photophase/day	0.34	0.31	0.31	0.18	0.08
Temperature at:					
Hatching	18.6	20.0	21.4	16.0	16.2
Pupation	17.8	15.6	17.4	19.2	15.6
Change in temperature from hatching to pupation	-0.8	-4.4	-4.0	+3.2	-0.6
Rate of change, C/day	0.04	0.22	0.20	0.15	0.03
Mean temperature of aquatic stages	19.5	18.3	18.8	17.0	17.1

Anopheles earlei at 24 C requires 18 days to develop from egg to adult (342 day-degrees above 5 C) and a further 6 days from eclosion to egg laying, (Kreutzer and Kitzmiller, 1969). For simplicity, however, I have assumed that *An. earlei*, *Culex territans* and *Culiseta alaskaensis* all spent 20 days in the larval stage and 10 days in the pupal and teneral stages combined. On this assumption, in 1974 the diapausing *An. earlei* hatched on 14/vii and pupated on 3/viii, and in 1975 they hatched on 9/vii and pupated on 29/vii. The daylength would have been 18:20 to 18:36 at hatching and 17:06 to 17:26 at pupation, which represents a decrease of 1:10 to 1:14, or 3.5 - 3.7 min/day, between hatching and pupation, (Table 39). The temperature decreased by 0.8 C between hatching and pupation in 1974 and 4.4 C in 1975. The mean temperatures during the aquatic stages, however, were quite close, 19.5 C in 1974 and 18.3 C in 1975.

Most of the *Clx. territans* were collected on windows on the U of A campus. None were collected between mid-July and late August, 1974, so it is not possible to fix the date of appearance of the diapausing females in that year. In 1975 the last gravids were taken in late July; the date of appearance of diapausing females was taken as 5/viii. All the unfed females taken in early July 1974 were nulliparous and apparently in diapause, but are considered to be very recent post-tenerals, since gonoactive, fed and gravid females were taken after that date. The results on which these conclusions are based are shown in Section 3.9.

The diapausing females in 1975 are estimated to have hatched on 6/vii, when the daylength was 18:42, and to have pupated on 26/vii when it was 17:33. Thus they would have experienced a decrease in daylength of 1:09, or 3.4 min/day, between hatching and pupation, (Table 39). The temperature decreased by 4.0 C between hatching and pupation, and the mean temperature of the aquatic stages was 18.8 C.

Although *Cs. alaskaensis* females were taken at bait until early August, no nullipars were taken after May, long before the summer generation appeared. Thus diapause appears to be obligate in this species, but the timing and conditions of its appearance are still of interest. Most of the females were taken on windows on the U of A campus.

Two nullipars were taken in mid-June in 1974, but it was not determined if they were diapausing. Many nullipars were taken in late June and all were diapausing. In 1975, the first nullipars were taken in early July and all were diapausing, (see Section 3.10). The estimated dates of hatching were 26/v/74 and 5/vi/75, and the dates of pupation 15/vi/74 and 25/vi/75, (Table 39). Over this period the daylength in both years showed a slight net increase. In 1975 an increase was followed by a slightly smaller decrease, since the period crossed the summer solstice.

5.6. Discussion

Diapausing female *Cs. inornata* appear in mid-August when the daylength, including civil twilight, is around 16:15, and I have calculated that induction would have taken place at a still greater daylength. *Cs. inornata* reared in the laboratory at constant daylengths of 12:00 or 16:00 did not diapause, but diapause was induced by transfer from 16:00 to 12:00 at pupation, (Chapter 6). This single-step decrease in daylength, 4 hours in 20 days of development, or about 12 minutes per day, is about three times the decrease in daylength that the wild *Cs. inornata* would have experienced.

Thus we have to explain the discrepancy between laboratory and field observations. If a decrease in daylength is the controlling factor, then it still acts when both the initial and the final daylengths are greater than 16 hours. Danilevskii (1965) reports that pupal diapause may be introduced in *Acronycta rumicis* (Noctuidae), by constant daylengths, below a critical value which ranges from 14:30 for a population from Abkhazian (43° N), to over 19 hours for a population fron Leningrad (60° N). Decreases in daylength as rapid as 10 minutes per day failed to induce diapause when they did not go below the critical constant daylength. The "beginning of the mass appearance" of diapausing female *Anopheles maculipennis messeae* at Leningrad is 8/viii when the daylength is 18:10, and during the middle of their larval development the daylength was 19:44. In laboratory experiments with Leningrad female mosquitoes reared under constant daylengths, however, only about 60 % diapaused when the daylength was 16 or 18 hours, but nearly all diapaused when it was 15 hours, (Vinogradova, 1960).

Since the rate of change of daylength increases from a minimum at the solstices to a maximum at the equinoxes, it is conceivable that diapause could be regulated in nature by an increase in the rate of change of daylength. The mechanism involved would have to be able to distinguish between 2.75 min/day (July 17 - 21) and 3.75 min/day (August 5 - 9).

The peaks of *Cs. inornata* females in light traps, on windows and at bait cattle, were in late July and early August, at least ten days after the diapausing generations are estimated to have hatched. Since the wave of diapausing females which appear at the end of August are likely to be descendents of the wave that appears in July, the life span of the diapausing generation appears to have been overestimated.

Even the last *Cs. inornata* collected before winter had undeveloped follicles with F:G ratios no greater than 1.5, but some *An. earlei*, *Clx. territans*, and *Cs. alaskaensis* in August had well-developed follicles. On the other hand, there was no sign of blood-feeding in fall by any of these species (Chapter 3). It may be that short or decreasing days prevent blood feeding but do not entirely prevent follicle growth. Tauber and Tauber (1976) give several examples of insects which require short or decreasing days to remain in diapause.

Edmonton (Namao Airbase) had a mean of 1470 day-degrees above 5 C per year during 1941 - 70, (unpublished data from Environment Canada , Edmonton). This would have been enough for 3 generations of *Cs. inornata*, but the second would have emerged some time in August and the third not until October, too late to accumulate any food reserves for winter. A cessation of reproduction in August is therefore a good adaption to the annual temperature cycle in the Edmonton region.

6. INDUCTION OF DIAPAUSE IN LABORATORY-REARED FEMALES

Experiments with wild-caught diapausing females gave inconsistent results (Chapter 4), possibly because of variation in the ages of the females. For any experiments on the physiology of diapause it is desirable to have diapausing females of known age. This chapter describes experiments to find a reliable method of inducing diapause in the laboratory.

6.1. Experiment 1: the effects of constant daylengths and temperatures on blood feeding and assimilation in *Culiseta inornata*.

6.1.1. Materials and methods

Two daylengths and two temperatures were investigated. The experiments began with first-instar larvae from the Edmonton I colony (Section 2.14.), and the mosquitoes were reared and maintained in two Sherer 25-7 incubators. Each incubator was lit by four 40 W fluorescent lamps, giving an illuminance of 4465 lux. The incubators got about 1 degree warmer when the lamps went on. Larvae reared at 16 hr/20 C and 8 hr/20 C were taken from the pooled hatch of 25 egg rafts in March 1973, larvae at 8 hr/10 C were reared from July to September 1973, and larvae at 16 hr/10 C from November to December 1973.

The larvae were reared in plastic trays with a maximum of 250 larvae and 2 litres of Bates' Medium per tray; each week surviving larvae were transferred to a clean tray. The larval diet was bread crumbs, powdered yeast and Difco Brain-Heart infusion, as used by McLintock (1952). Pupae were transferred to deionized water in 200 ml plastic cups covered with netting and a few cork slices on the water surface. Adults were kept in plastic cups covered with netting, with a filter paper disc on the bottom and wrapped in moist paper towels and transparent polyethylene sheets. Blood-feeding exposures were in a 250 ml glass beaker covered with netting placed on my forearm for 15 minutes. The first exposure was on the day after emergence, and daily thereafter until the females fed, died or (a few) escaped. Mosquitoes which had been exposed three times were pooled for later exposures. In all the experiments, females with any blood visible externally were considered blood-fed. Females offered blood received no other food. Blood-feds were inspected daily and dissected when the gut appeared empty, usually 6 days after feeding at 20 C and 14 days at 10 C. Some females at 10 C were never offered blood, given only cotton balls soaked in 10 % sucrose, and dissected 14 days after emergence. A few of the 8 hr/10 C group were dissected on the day of emergence.

6.1.2. Results

When the larvae that were supposed to be at 16 hr/10 C had nearly pupated it was found that the temperature in the trays was 12 C. The incubator was reset, but the larvae had developed more rapidly than those at 8 hr/ 10 C (Table 40, col. 2 vs. col. 1). When the two incubators were set to 20 C, the mean development times to adult emergence were much closer. Median development times for the females, calculated from the same data, are shown in Chapter 5. Mortality of the larvae was high at 10 C, most of the deaths occurring in the early instars.

Table 40. Diapause induction experiment 1: development time and mortality of *Cs. inornata*.

Daylength (hr)	8	16	8	16
Temperature (C)	10	10-12	20	20
Number of larvae at start	400	500	400	400
% mortality: larvae	69.8	73.8	22.3	14.5
pupae	1.3	4.2	8.0	9.8
Total	71.1	78.0	30.3	25.3
Mean time from hatching to adult emergence(days):males	58.0	48.4	19.8	21.4
females	69.2	54.0	21.3	23.3

More than half the females under both daylengths at 20 C took blood at the first or second exposure, and about 85 % altogether, (Table 41). None of the females reared at 10 - 12 C took blood at the first exposure, and most died without feeding, the total feeding rate for the 16 hr treatment (42.1 %) being slightly higher than for the 8 hr treatment (33.3 %). More than 80 % of the blood-fed females at 20 C matured eggs, including those that fed only 1 day after eclosion, (Table 41). Only 50 % of the blood-feds reared at 16 hr/10 - 12 C and 27 % of those reared at 8 hr/10 C, both small samples, showed any egg development. The average number of eggs was lower in females reared at 10 - 12 C than in those reared at 20 C, (Table 42). A few of the blood-feds in each treatment developed substantial fatbodies; these were mostly the females that did not mature eggs but some had both and others neither. Six newly emerged females at 10 C had no visible fat deposits, but all those given 10 % sucrose developed fatbodies. One of the sugar-fed 8 hr/10 C females was autogenous with 49 stage IV follicles. Insemination rates were 91 - 100 % in the 4 treatments.

6.2. Experiment 2: the effects of a decrease in daylength and temperature at different stages of development on follicle growth in *Cs. inornata*.

The results of experiment 1 were hard to interpret because of the small numbers of blood-feds at 10 C, due to high larval mortality and low blood-feeding rates. Since Vinogradova (1960) and others were able to induce diapause in female mosquitoes by subjecting them to a decrease in daylength at the third or fourth larval instar,

Table 41. Diapause induction experiment 1: blood-feeding rates of *Cs. inornata*, and numbers of blood-feds maturing eggs.

Rearing and holding regime		Number exposed	Blood-fed		(d) Number dissected	Mosquitoes with folls >IIb	
			No.	%		No.	% of d
16 hr/20 C	1st exposure	139	45	32.4	42	39	92.8
	2nd exposure		51	36.7	45	43	95.6
	3rd exposure		14	10.1	10	10	100
	Later exposures		7	5.0	4	3	75
	Total	139	117	84.2	101	95	94.0
8 hr/20 C	1st exposure	118	23	19.5	23	14	60.9
	2nd exposure		55	46.6	54	48	88.9
	3rd exposure		12	10.2	11	9	81.8
	Later exposures		11	9.3	9	8	88.9
	Total	118	101	85.6	97	79	81.4
16 hr/10-12 C	1st exposure	38	0	0	0	0	0
	2nd exposure		0	0	0	0	0
	3rd exposure		0	0	0	0	0
	Later exposures		16	42.1	16	8	50
	Total	38	16	42.1	16	8	50
8 hr/10 C	1st exposure	39	0	0	0	0	0
	2nd exposure		1	2.6	1	1	100
	3rd exposure		2	5.1	2	0	0
	Later exposures		10	25.6	8	2	25
	Total	39	13	33.3	11	3	27.3

Table 42. Diapause induction experiment 1: Assimilation of blood and sugar meals by *Cs. inornata* females.

Daylength (hr) Temperature (C)	8 10	16 10-12	8 20	16 20
Blood-fed				
No. examined	11	16	97	101
% with follicles > IIb	27.3	50.0	81.1	94.0
Mean eggs/female	67.0	80.1	93.2	93.4
% with fatbody 2 or 3	18.4	31.3	18.6	7.0
10% sucrose ad lib 15-21 days				
No. examined	11	6	0	0
% with follicles > IIb	9	0	-	-
No. of eggs/female	49	-	-	-
% with fatbody 2 or 3	100	100	-	-

/

it was worth investigating whether diapause could be induced in larvae which had spent their early instars at 20 C in the culture room. In experiment 2, follicle development rather than gonotrophic dissociation was used as a criterion for diapause.

6.2.1. Materials and methods

The experiment began with first-instar larvae from the third and fourth generations of the Edmonton II colony. Larvae were reared in the culture room at 16 hr/20 ± 2 C or in an incubator at 12 hr/10 ± 1 C, on a 2:1 v/v mixture of laboratory rabbit chow and bakers' yeast. Adults were kept in cages 2.5 x 2.5 x 15 cm (i.d.) with sides of plexiglas and tops and bottoms of nylon tulle, (Trpis, 1968). Two soaked raisins were placed on each cage, and the cages were kept over wet paper towels in plastic trays covered with glass. To each batch of females was added an equal or slightly smaller number of males, usually their siblings. Many adults stuck in condensation on the sides of the cages and died. Other techniques were described for experiment 1, and in Section 2.14.

Two constant and three changed daylength and temperature regimes were investigated, as follows:

(a) Transferred from the culture room (16 hr/20 C) to an incubator at 16 hr/20 C on the day of pupal-adult ecdysis (dummy transfer);

(b) Reared and maintained from egg-hatching onwards in the 12 hr/10 C incubator;

(c) Transferred from the culture room to 12 hr/10 C incubator on the day of ecdysis to the fourth larval instar;

(d) Transferred from the culture room to the 12 hr/10 C incubator on the day of ecdysis to the pupa;

(e) Transferred from the culture room to the 12 hr/10 C incubator on the day of pupal-adult ecdysis (eclosion).

The culture room and incubators were lit by single 15 W cool white fluorescent lamps, giving illuminance values of 12 and 807 lux, respectively, at the larval rearing trays. Thus the larvae that were transferred experienced decreases in daylength but increases in illuminance.

At two-day intervals from adult emergence through to 16 - 17 days later, five females from each treatment were dissected and follicles in 0.7 % NaCl under a cover glass were examined, with phase contrast at X 400. Other dissection techniques, and follicle stages were as described in Sections 2.6. and 2.7. The lengths of ten follicles and their germaria were measured for each female. Mosquitoes were not allocated to the different treatments at random. Instead material was reared under each regime in excess of what was needed, and the females of the required ages removed on days convenient for dissection. This meant there was a greater chance of females being siblings within treatments than between treatments. Follicles for measurement were also not selected at random, since preference was given to those undistorted by pressure from the cover glass or from adjacent follicles.

6.2.2. Results

The follicles of the females reared and maintained at 16 hr/20 C reached stage II by 4 - 5 days after emergence, (Table 43), when the mean F:G ratio had already exceeded 2.4, and showed only a

Table 43. Diapause induction experiment 2: follicle stage distribution of *Cs. inornata* females from 0-16 days after emergence. Day 14-15: n = 50, other days: n = 5.

	Time (Days) after emergence								
	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15[(x)]	16-17
(a) Reared and maintained at 16 hr/20 C.									
N1	4								
N2	1								
I									
I-II		5	2	1	2			10	
IIa			3	3	3	4	2		4
IIb				1		1	3	40	1
(b) Reared and maintained at 12 hr/10 C.									
N1	1								
N2	1								
I	3	5	4	4	5	4	3	11	
I-II			1	1		1			
IIa							2	28	2
IIb								11	3
(c) Transferred to 12 hr/10 C at IV instar.									
N1	5								
N2									
I		5	5	5	5	5	5	40	3
I-II								10	2
(d) Transferred to 12 hr/10 C at pupation.									
N1	1								
N2	4								
I		5	4	5	5	5	3	36	4
I-II							2	14	1
(e) Transferred to 12 hr/10 C on day of emergence.									
N1									
N2			2						
I	5	5	3	5	5	2	4	28	5
I-II						2	1	12	
IIa						1		10	

(x) For graphic representation of distributions at 14-15 days see Fig. 64.

slight increase after that, (Fig. 63). In the females transferred at the IVth instar or at pupation, no stage II follicles were seen, and the mean F:G ratios never exceeded 1.80 in either group. Of the females transferred at eclosion, one at 10 - 11 days and one at 14 - 15 days (Fig. 64) had stage II follicles; the mean F: G ratio was 1.8 at 2 - 3 days post-emergence and stayed between 1.8 and 2.0 until the end of the experiment. In the females reared and maintained at 12 hr/10 C some stage II follicles were seen at 12 - 13 days. At 14 - 15 days most of the follicles were in stage II, and the F:G ratio had reached 2.51, almost as high as in the females kept at 16 hr/20 C throughout. Two autogenous females were seen. One of the females maintained at 16 hr/20 C throughout had 27 mature eggs at 12 - 13 days after emergence. One of the females transferred to 12 hr/10 C at emergence had 52 stage III follicles in one ovary at 16 - 17 days post-emergence, and the other ovary could not be found, which suggests that it had atrophied, and was certainly not as advanced as stage III. Neither of these mosquitoes was used in calculating F:G ratios; extra mosquitoes were dissected instead.

The F:G ratios at 14 - 15 days were subjected to a nested analysis of variance, the results of which are shown in Table 44. In spite of the significant difference between mosquitoes within treatments, the difference between treatments was highly significant ($p < 0.01$). The results were further analysed by Duncan's Multiple Range Test (Steel and Torrie, 1960), using "mosquitoes in treatments" as the error term. There was a highly significant difference ($P < 0.01$) between the means for the mosquitoes reared under constant

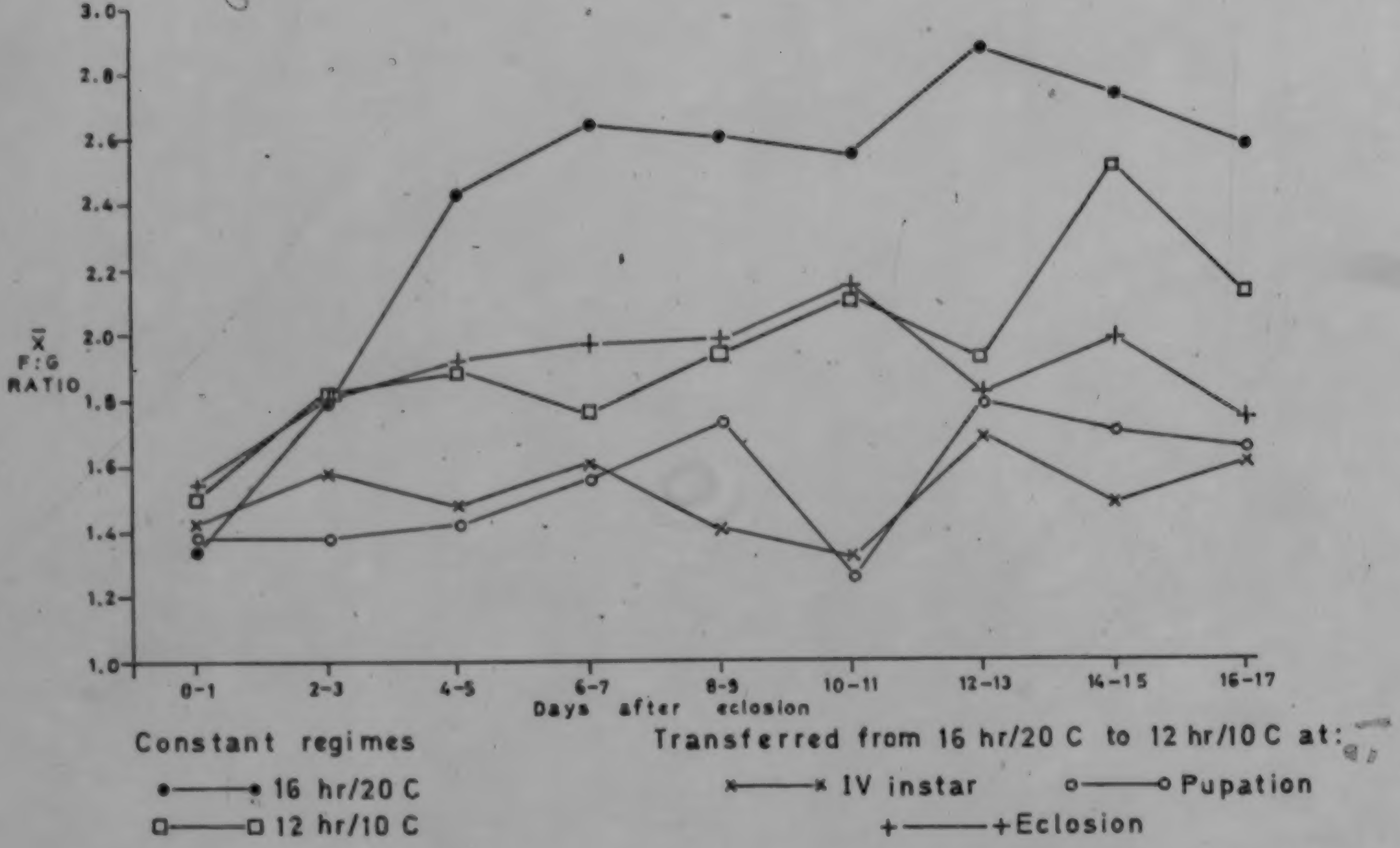


Fig. 63. Diapause induction experiment 2: mean F:G ratios of *Culiseta inornata* at 2-day intervals from 0-16 days after eclosion. n = 5 females x 10 follicles = 50 per point.

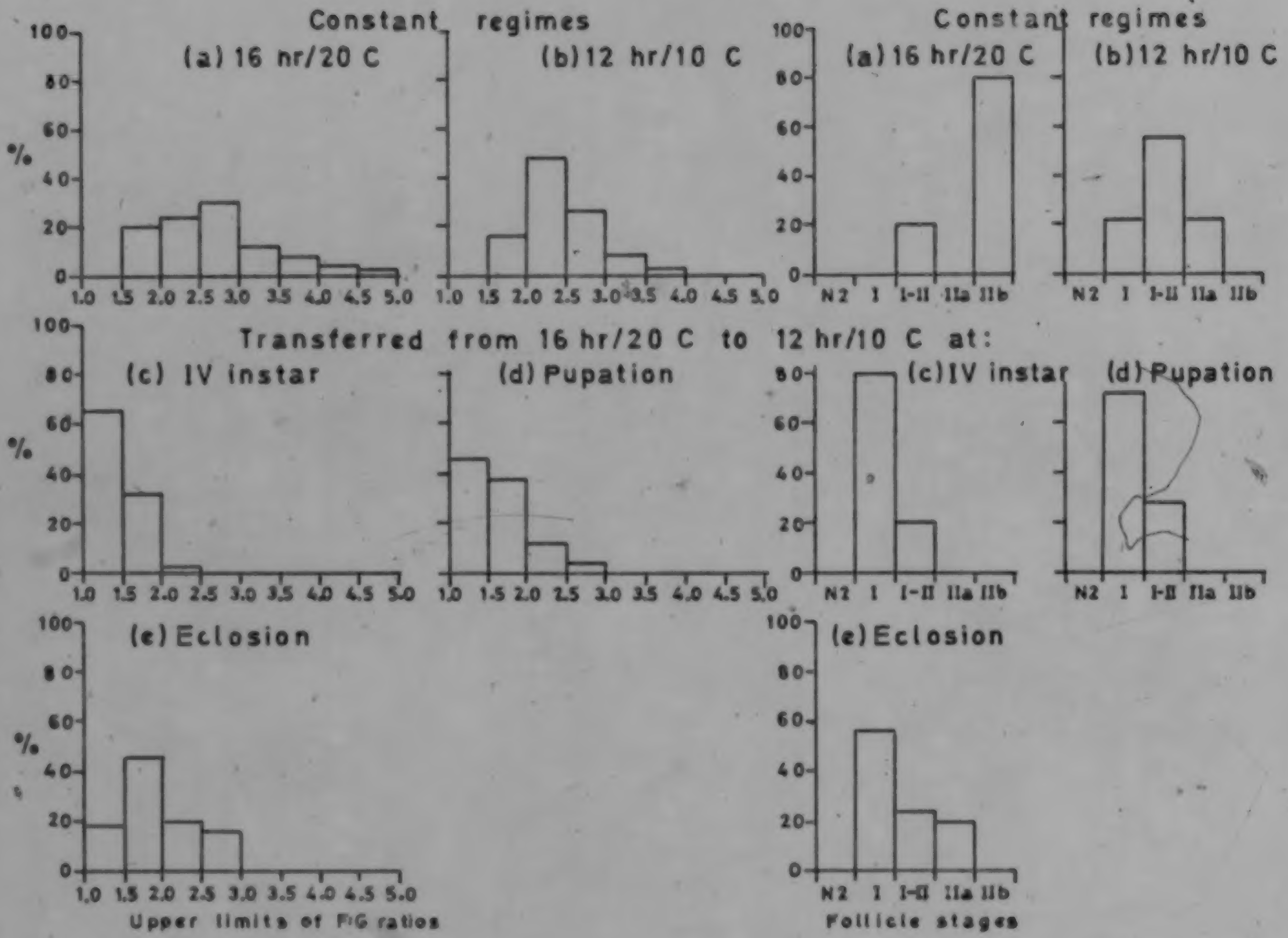


Fig. 64. Diapause induction experiment 2: distribution of F:G ratios and follicle stages in *Cs. inornata* at 14-15 days after eclosion. n = 5 females x 10 follicles = 50.

conditions and the means for the mosquitoes transferred at different stages of development (Table 45), but there were no significant differences within these two groups. In the same table the data on rates of diapause are summarised. Using the criterion of F:G ratio, only the females transferred at the IVth instar had more than half their F:G ratios 1.5 or less, but none of the follicles of those transferred at pupation and only 20 % of the follicles of those transferred at emergence had reached Stage II.

The insemination rates are shown in Table 46. The rates for three of the treatments were low and caused concern that the differences in follicle development might have been an indirect result of some effect of the treatments on insemination. Although there were differences between the mean F:G ratios of inseminated and uninseminated females they were not all in the same direction, andwere not large enough to account for the differences between treatments. Insemination rates for the other two treatments were over 95 % and were not analysed further.

6.3. Experiment 3: the effects of decreases in daylength and temperature at pupation on follicle development, blood-feeding and assimilation in *Cs. inornata*

The results of experiment 2 indicated that diapause could be induced by a decrease in daylength and temperature,at ecdysis to the fourth instar,or at pupation. Experiment 3 had two objectives: to find if both the decrease in temperature and the decrease in daylength were essential, and to compare follicular development with

Table 45. Diapause induction experiment 2: ranked mean F:G ratios and numbers of *Cs. inornata* in diapause 14-15 days after emergence.

	Constant				Changed from 16 hr/20 C to 12 hr/10 C at					
	16 hr/20 C		12 hr/10 C		Emergence		Pupation		IV Instar	
Mean F:G ratio	2.72	=	2.51	>	1.98	=	1.70	=	1.48	$p < 0.01$
% of F:G ratios no greater than 1.50	0		0		18		46		66	
% of follicles earlier than stage II	20		22		80		100		100	

Table 46. Diapause induction experiment 2: insemination rates and mean F:G ratios of inseminated and uninseminated *Cs. inornata*.

Numbers inseminated, out of 5 (unless otherwise indicated).

Age (days)	Constant regimes (a) 16 hr/20 C	Constant regimes (b) 12 hr/10 C	Transferred from 16 hr/20 C to 12 hr/10 C at: (c) IV instar	(d) pupation	(e) eclosion
0-1	0	0	0	2	3
2-3	5	2(4)	4	5	4
4-5	5	1	5	4	2(4)
6-7	4(4)	3	4	2	3
8-9	5	5	4	4(4)	4
10-11	5	5	5	3	4
12-13	5	5	5	3	5
14-15	4(4)	5	5	4	4
16-17	3	4	5	4	5
Total examined(x)	38	39	40	39	39
Number inseminated	36	30	38	29	31
% inseminated	95	77	95	74	79

Mean F:G ratios of inseminated and uninseminated females.

Age (days)	Group b Insem.	Group b Uninsem.	Group d Insem.	Group d Uninsem.	Group e Insem.	Group e Uninsem.
2-3	1.85	1.76	-	-	1.81	1.80
4-5	1.78	1.91	1.77	1.30	1.88	1.93
6-7	1.85	1.54	1.59	1.51	1.82	2.17
8-9	-	-	-	-	1.96	1.94
10-11	-	-	1.31	1.15	2.19	1.98
12-13	-	-	1.98	1.54	-	-
14-15	-	-	1.71	1.66	1.90	2.26
16-17	2.16	1.97	1.66	1.54	-	-
Mean	1.91	1.80	1.67	1.45	1.93	2.01

(x) 0-1 days excluded.

blood-feeding and assimilation.

6.3.1. Materials and methods.

Second-generation descendants of gravid females collected from the windows in June and July 1975 were reared in the culture room at 16 hr/20 C using the same methods as in experiment 2 and transferred at pupation to plywood boxes (Section 2.14.) in incubators at 10, 15 and 20 C. Each box had two 6 v incandescent lamps giving illuminance values of 54 - 86 lux, and there were two boxes in each incubator, one with a daylength of 12 hr and one with 16 hr. Temperatures in the boxes were within 1 Celsius degree of those in the incubators outside. Each day's pupation was pooled and redivided into six groups allocated at random to the different treatments. Batches of 10 - 20 adults were kept in plastic pill vials 10 cm long x 4.3 cm in diameter, lined with paper towelling and filter paper and covered with gauze, in the same boxes as the pupae. A soaked raisin, changed daily, was placed on each vial, and removed 2 days before feeding exposures.

For feeding exposures, females were transferred to the plastic cages covered on two sides with netting, (see Section 6.2.1.). Several cages at a time were held against my forearms with cylinders cut from stretch nylon stockings. There were three daily exposures of 15 minutes each, starting on day 7 after emergence. Any females which had not fed after three exposures were discarded. The room temperature during feeding exposures was 23 - 24 C, and the illuminance under the stocking used to hold the cages was 516 lux. Blood-fed.

females were transferred to plastic cups covered with netting, and provided with water-soaked cotton balls, and returned to the boxes they were taken from. After 7 days (at 20 and 15 C) or 14 days (at 10 C) the blood-fed females were dissected and examined for egg development and insemination.

From each treatment ten mosquitoes that had been fed raisins only were dissected 14 - 15 days after emergence, and 10 follicles from each female were measured and assigned a stage, using the methods described for experiment 2.

6.3.2. Results

In all females kept at a daylength of 12 hr, no more than 10 % of the follicles had reached stage II by 14 - 15 days after emergence, but in the females kept at a daylength of 16 hours, 70 - 100 % of the follicles had reached stage II, (Fig. 65). By 14 - 15 days after emergence, 33 - 50 % of the F:G ratios in the 12 hr groups and 92 - 100 % of the F:G ratios in the 16 hr groups were greater than 1.5. There was a highly significant difference between the F:G ratios for the different treatments, (Table 47). Duncan's multiple range test shows a highly significant difference between the mean F:G ratios for the two daylengths but no significant difference between the means for different temperatures at the same daylength, (Table 48). Note that in Table 48 the mean F:G ratio for 16 hr/20 C is ranked lower than for 16 hr/15 C.

For each daylength the proportion of females taking blood increased with temperature, (Table 49), and for each temperature the

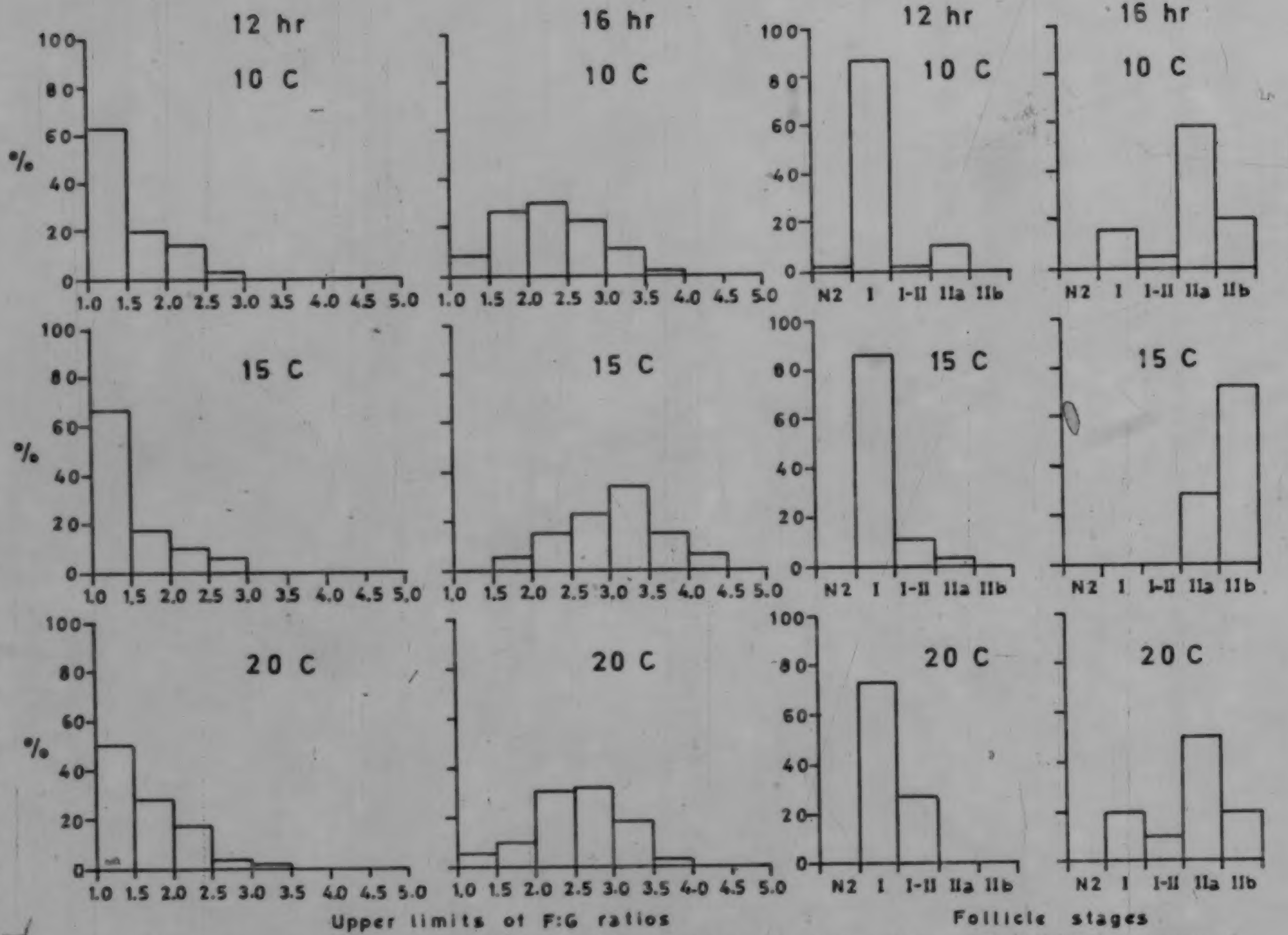


Fig. 65. Diapause induction experiment 3: distribution of F:G ratios and follicle stages in *Ca. inornata* 14-15 days after eclosion.

Table 47. Diapause induction experiment 3: nested analysis of variance in F:G ratios of *Cs. inornata* 14-15 days after emergence.

Source of variation	df	SS	MS	F	F at 0.01
Treatments	5	218.81	43.76	22.10**	4.76
Mosquitoes in treatments	54	107.05	1.98	16.50**	1.73
Follicles in mosquitoes	540	64.75	0.12		
Total	599	390.61			

** = Significant at $p < 0.01$

df = degrees of freedom

SS = sum of squares

MS = mean square

Table 48. Diapause induction experiment 3: ranked mean F:G ratios, and percentages of diapause-stage follicles in *Cs. inornata* 14-15 days after emergence.

Daylength (hr)	12						16					
Temperature (C)	10		15		20		10		20		15	
Mean F:G ratio	1.51	=	1.52	=	1.66	<	2.31	=	2.61	=	3.10	$p < 0.01$
Percentage of F:G ratios no greater than 1.50	63		67		50		8		5		0	
Percentage of Follicles earlier than stage II	100		97		90		30		21		0	

Table 49. Diapause induction experiment 3: blood-feeding and assimilation in *Cs. inornata* and expected percentages maturing eggs.

Photoperiod (hr)	12			16		
Temperature (C)	10	15	20	10	15	20
Feeding						
Number exposed	66	78	47	66	68	56
Percent fed	20	42	62	35	72	66
Assimilation						
Number of feds examined	13	33	29	22	46	37
Percent with eggs	0	12	55	86	96	100
Expected percent with eggs						
From % of Foll:Ger ratios >1.50	37	33	50	92	100	95
From % of Follicles in stage II	0	3	10	70	100	79

blood feeding rate was higher at the longer daylength. At 16 hour daylength 86 - 100 % of the blood-feds matured eggs; at 12 hour the egg maturation rate was lower and more variable, 0 - 55 %. There was no exact correspondence between the percentages of blood feds maturing eggs and the follicular development of the raisin-fed females. The expected percentages maturing eggs (last 2 lines of Table 49) are based on the assumption that only females with follicles in stage II or F:G ratios greater than 1.50 at 14 - 15 days after emergence would mature eggs. In general, the percentages of females maturing eggs were lower than would have been expected from the F:G ratios and higher than would have been expected from the percentages with follicles in stage II. Although only 60 females were measured at 14 - 15 days after emergence, 90 were dissected to search for autogeny. Two autogenous females were found, both in the 16 hr/20 C group; one had 27 stage V follicles, and the other had 6 stage V and 4 stage III follicles. All the females examined, both raisin-fed and blood-fed were inseminated.

6.4. Experiment 4: follicle growth, blood-feeding and assimilation in *Culiseta alaskaensis* at 16 hr/20 C

Blood-fed females collected from the Egyptian trap at George Lake in May, 1975 were kept in the laboratory until they laid eggs. The larvae were reared at 16 hr/20 C in the culture room using the same diet and methods as for *Culiseta inornata*. Females were kept in vials in boxes in an incubator at 16 hr/ 20 C, as in experiment 3, and given raisins. Some females were dissected every 2 days from emergence until 14 - 15 days, and at 40 - 43 days. Others were deprived of raisins from 5 - 7 days onwards and exposed to my arm on 6 occasions, the first at 8 - 10 and the last at 27 - 29 days after emergence. Blood-feds were dissected 7 days after feeding. No males were put with the females because no matings had ever been seen in a 1 ft^3 cage where other males and females were housed. The follicles of the unfed females grew very slowly, (Table 50). The first in stage I were seen at 8 - 9 days after emergence, and none had reached stage II by 40 - 43 days. The mean F:G ratio was well below 1.5 at 14 - 15 days but 1.83 at 40 - 43 days.

Fifty per cent of the females had fed after 4 exposures (Table 51), but the meals were small. None of the blood-feds produced mature eggs, but in 5 out of 9 the follicles reached stage II or III, a feature not seen in any of the raisin-fed females. Although mating may have occurred in the emergence cage before the females were transferred to the tubes, none of the females were inseminated.

6.5. Discussion

The results of experiment 1 suggested that temperature was a more important factor than daylength. However, Mer (1936) found than in *Anopheles sacharovi* blood-fed females did not mature eggs unless the follicles had reached stage II before feeding. In experiment 1 the feeding exposures began on the day after adult emergence. Since most of the females at 10 - 12 C did not feed until after the third exposure, their ages at feeding are not known, but were probably not more than 10 days since most had died by then.

Table 50. Diapause induction experiment 4: follicular development in uninseminated *Cs. alaskaensis* at 16 hr/20 C. n = 5 mosquitoes, 1 follicle per mosquito.

Time from emergence (days)	Mean Follicle:Germarium ratio	Nos. in Stage			Number in diapause
		N1	N2	I	
0-1	1.29(2)*	3	2	0	5
2-3	- (0)	5	0	0	5
4-5	1.22	1	4	0	5
6-7	1.25	0	5	0	5
8-9	1.26	0	2	3	5
10-11	1.18	0	0	5	5
12-13	1.13	0	0	5	5
14-15	1.13	0	0	5	5
40-43	1.83	0	0	5	1

* The numbers in parentheses are the numbers measured; the other mosquitoes had not yet formed follicles.

Table 51. Diapause induction experiment 4: blood-feeding and assimilation by 20 uninseminated *Cs. alaskaensis*, reared and held at 16 hr/20 C.

Attempt	Age (days)	Number fed	Number dissected	Stage					$\bar{x}$ F:G ratio	Number in diapause
				I	I-II	IIa	IIb	III		
1	8-10	2	1	0	0	0	1	0	2.67	0
2	13-15	1	1	0	0	0	1	0	2.67	0
3	17-19	5	5	3	0	1	0	1	1.80	3
4	21-23	2	2	0	1	1	0	0	2.16	0
5	24-26	0	0							
6	27-29	0	0							
Totals		10/20 = 50%	9	3	1	2	2	1	2.32	3/9 = 33%

Since the follicles did not reach stage II until 12 - 13 days after emergence in mosquitoes reared and maintained at 12 hr/10 C, (experiment 2) the blood-fed mosquitoes at 10 - 12 C in experiment 1 could have failed to mature eggs because their follicles were not ready at the time of feeding. On the other hand, the majority of females at 20 C which took blood on the day after emergence matured eggs, (table 41), though their follicles would barely have reached stage I at the time of feeding, (Tables 34 and 43).

A few blood-fed females in all treatments in experiment 1 had well-developed fatbodies but no eggs, that is, they showed true gonotrophic dissociation (Table 42). The actual fat content was not measured, and some of it may have been derived from the larval muscle remnants, but most of the females which matured eggs did not have well-developed fatbodies. Kalpage (1970) reared *Cs. inornata* females at various constant temperatures between 10 and 30 C and constant daylengths of 8, 12 and 16 hr, and offered them his blood 5 days after they emerged. Blood-feeding rates increased with daylength and with temperature, as in my experiments 1 and 3, but were lower than in my experiment 1 (shown in parentheses), as follows: 8 hr/10 C, 18 % (33); 16 hr/10 C, 30 % (42); 8 hr/20 C, 32 % (86); 16 hr/20 C, 60 % (84). Kalpage probably obtained lower feeding rates because he only offered them blood once. On the other hand far more blood-feds at 10 C matured eggs in Kalpage's experiment than in mine, (in parentheses): 8 hr/10 C, 80 % (27); 16 hr/10 C, 70 % (50); 8 hr/20 C, 75 % (81); 16 hr/20 C, 88 % (94). The higher egg maturation rates in Kalpage's treatments at 10 C may have been because his females were older than mine when they fed, but the differences could equally well have been due to chance since his sample sizes (5 at 8 hr and 10 at 16 hr) were even smaller than mine.

In experiments 1 and 3 fewer females kept at 10 C took blood than did those kept at 15 or 20 C, in spite of the fact that all feeding exposures took place at around 23 C. Many *Cs. inornata* females were taken on cattle at George Lake at 7 C on 6/viii/74 (see Fig. 37). It may be that a sudden change of temperature in either direction will inhibit feeding until the females have acclimated.

In experiment 2, 20 % of the follicles of females transferred to 12 hr/10 C in the IVth larval instar, and 28 % of the follicles of those transferred at pupation, were in stage I-II, though almost all the F:G ratios in these treatments were less than 1.5. These females may still be considered in diapause because some stage I-II follicles were also found in wild-caught diapausing females. Twenty females collected from 1 - 10/ix/75 were first measured under the dissecting microscope at X 150 (1 typical follicle per female) then reexamined and measured with phase contrast at X 400 (5 follicles per female). At X 150 all follicles were classed at stage I, with a mean F:G ratio of 1.22 ± 0.06, but at X 400, 24 % of the follicles were classed as stage N2, 62 % as stage I and 14 % as stage I - II, and the mean F:G ratio was 1.29 ± 0.03.

A decrease in daylength from 16 to 12 hr at the IVth instar or at pupation induced diapause in *Cs. inornata* (experiments 2 and 3), but rearing throughout at 12 hr/10 C did not, (experiment 2). This suggests that *Cs. inornata* is a long day-short day (LD/SD) animal. This response is known in some other insects (Tauber and Tauber, 1973a), mammals, birds (Andrewartha and Birch, 1954) and plants (Salisbury and Ross, 1969). However, the possible effects of constant short days should not be dismissed without further investigation, since several factors could have masked the response. A daylength of 12 hours may be too short to produce the maximum diapause response in *Cs. inornata*. In *Grapholitha molesta*(= *Laspeyresia*) (lepidoptera: Olethreutidae), most larvae diapaused at daylengths of 10 to 14 hr, fewer diapaused at less than 10 hr, (Dickson, 1949). There are many examples of the critical daylength for diapause induction being affected by temperature, and the photoperiodic reaction may be suppressed by both high and low suboptimal temperatures. In *Acronycta rumicis* (Lepidoptera: Noctuidae) the critical daylength increased by 1 1/2 hour with each 5 C fall in temperature (Danilevskii, 1965). It may be temperatures of both 20 and 10 C suppress the response to daylength in *Cs. inornata*, though it seems unlikely because such temperatures were frequently recorded in the lakeside pond at George Lake in mid-July and late August, respectively, (Chapter 5).

Tauber and Tauber (1973a) distinguish three types of photoperiodic induction of diapause in insects, as follows:

a) Induction by constant daylengths above or below a critical value. This type has been demonstrated in several mosquito species, including *Anopheles maculipennis messeae*, (Vinogradova, 1960), *An. freeborni* (Depner and Harwood, 1966), and *Culex pipiens* (Sanburg and Larsen, 1973; Spielman and Wong, 1973b).

b) Induction by changing daylengths which cross a certain critical value. I know of no demonstrations of LD/SD responses in mosquitoes other than *Culiseta inornata*, but it is interesting that diapause induction in *Culex tarsalis* was more consistent in females transferred from constant light to 8 hr days at pupation than in females reared at constant 8 hr daylengths (Harwood and Halfhill, 1964, Fig. 1 and 2, respectively). In *Nomadacris septemfasciata* (Orthoptera: Acrididae) the adults diapause if exposed to daylengths of less than 12 hr, but the diapause is intensified if the hoppers have experienced longer days, and suppressed if they have experienced shorter days, (Norris, 1965). In *Heliothis zea* (Lepidoptera: Noctuidae), a few larvae diapaused at constant daylengths of less than 13 hr, but diapause rates over 90 % were attained only when the parents and eggs experienced daylengths of 13 hr, the larvae 11 hr, and there was a concurrent decrease in temperature, (Roach and Adkisson, 1970).

c) Induction by changing daylengths not crossing a critical value. Shipitsina (1959) noted that diapause in *Anopheles maculipennis* populations in different parts of the U.S.S.R. appeared when the daylengths had reached a fixed proportion of the maximum daylength for the region, and she suggested that the decrease rather than the absolute daylength was the cue for diapause. An alternative explanation for this phenomenon was provided by Vinogradova's (1960) demonstration of geographical races of *An. maculipennis*, responsive to different constant daylengths. Imaginal diapause appeared in *Chrysopa carnea* (Neuroptera: Chrysopidae) in nature in August, at daylengths of 13.9 - 14.8 hr, but the critical constant daylength for laboratory induction of diapause was 13.5 - 14.0 hr, corresponding to natural daylengths in September, (Tauber and Tauber, 1973b). The growth of *Coleophora laricella*, (Lepidoptera: Coleophoridae), larvae is suspended at constant daylengths of 12 or 16 hr, but pupation occurs when larvae are transferred from 16 to 12 hr (Ryan, 1975). This is the opposite of my results with *Cs. inornata*, where follicle development is arrested by a decrease in daylength.

Cs. inornata would have to be assigned to Tauber and Tauber's type (c) by default, since no constant daylength inducing diapause was demonstrated. The fact that diapause was induced by a decrease in daylength from 16 to 12 hr in the laboratory, and from approximately 18 to 16 hr in the field, (Chapter 5), suggests that change in daylength may be more important than absolute daylength. If a decrease in daylength was all that was necessary, however, we would expect any eggs hatching after the summer solstice to develop into diapausing adults, which we would expect to see about July 21. In fact, the mass appearance of diapausing females occurred around August 20, nearly 2 months after the summer solstice. This suggests that absolute daylength, or some more subtle factor such as rate of change, are also important in diapause induction.

The results for *Cs. alaskaensis* indicate that diapause is obligatory, unless 16 hr is a short day for this species. Uninseminated females of some other species fail to produce eggs after feeding, (Clements, 1963), and this factor may have affected the results for *Cs. alaskaensis*. The great stability of diapause in *Cs. alaskaensis* suggests that its response to daylengths may be similar to that of *Stenocranus minutus* (Homoptera: Delphacidae), in which full ovary development will not take place under constant long or short days, and only occurs when females are transferred from short to long days, (Thiele, 1973).

7. COLLECTION OF FEMALES FROM OVERWINTERING SITES

7.1. Introduction

Published records of overwintering sites of *Anopheles*, *Culex* and *Culiseta* species recorded from Alberta are shown in Table 52. My aim was to examine all the known overwintering sites, but I know of no caves or talus slopes in the study area, and 2 of the mammals whose quarters sometimes harbour mosquitoes, the wood rat, (Ryckman and Arakawa, 1952), and the marmot (Shemanchuk, 1965) occur in Alberta only in the Rocky Mountains (Soper, 1964). In southern Alberta Shemanchuk (1965) also found *Clx. tarsalis* and *Cs. inornata* in the burrows of badger, skunk, porcupine and coyote, but not in the burrows of Richardson's ground squirrel.

Except for two records of *Anopheles earlei* in unheated buildings above ground and one for *Culex territans* in a tree-squirrel nest, all the known overwintering sites are well insulated from the outside air, and most are under the snow or underground. This is probably a true record of distribution since the more exposed sites are much easier to search.

7.2. Sites searched and methods of collection

All mosquitoes described as "overwintering" were collected in the months of November through March. Mosquito movement between sites is unlikely in this season because temperatures are usually below 0 C. Most of the searching was done after January, the coldest month, to lessen the chance of obtaining mosquito records from sites

Table 52. Known overwintering sites of females of *Anopheles*, *Culex* and *Culiseta* species recorded from Alberta.

Species and Site	Region	Authors
Anopheles earlei		
Mammal burrows	Central Alberta	Shemanchuk (1965)
Shed	Southern Manitoba	McLeod and McLintock (1947)
Caves	Minnesota	Owen (1937)
Empty Buildings	Alaska	Hopla (1970)
Culex territans		
Between stones	Region not stated	Howard et al. (cited by Hearle, 1926)
Nests of Tree Squirrel (*Tamiasciurus*)	Alaska	Hopla (1965)
Clumps of grass (*Calamagrostis*)	Alaska	Hopla (1970)
Cellars	Massachusetts	Berg and Lang (1948)
Caves	Minnesota	Price et al. (1960)
Food Storage Cellars	Nebraska	Keener (1952)
Culex tarsalis		
Mammal burrows	Southern Alberta	Shemanchuk (1965)
Rockpiles	Washington	Rush et al. (1958)
Food Storage Cellars	Nebraska	Keener (1952)
Mines	Colorado	Blackmore and Winn (1956)
Mines	Utah	Dow et al. (1956)
Nests of Wood Rat (*Neotoma*)	Riverside Co., California	Ryckman and Arakawa (1952)
Talus slopes	Oregon	Rush (1962)
Talus slopes	Utah	Trent (1960)
Talus slopes	Washington	Harwood (1962)
Culiseta alaskaensis		
Burrows of Arctic Ground Squirrel (*Spermophilus*)	Alaska	Hopla (1970)
Clumps of Grass (*Calamagrostis*)	Alaska	Hopla (1970)
Cellars	Moravia, Czechoslovakia	Minar and Hadjkova (1966)
Tree Holes, Caves, Cellars	USSR	Gutsevich et al. (1971)
Hollow Logs	United States, unspecified	Dyar (1922)
Culiseta impatiens		
Under Floors of Cabins	Montana	Mail (1934)
Culiseta incidens		
Rockpiles	Washington	Rush et al. (1958)
Talus slopes	Oregon	Rush (1962)
Culiseta inornata		
Mammal burrows	South and Central Alberta	Shemanchuk (1965)
Under Cabins	Montana	Mail (1934)
Caves	Banff, Alberta	Mail (1934)
Nests of Wood Rat (*Neotoma*)	Riverside County, California	Ryckman and Arakawa (1952)
Cellars, basements,potato pits	Utah	Rees (1943) cited in Horsfall (1955)
Rockpiles *	Washington	Rush et al. (1958)
Talus Slopes *	Oregon	Rush (1962)
Shafts	Utah	Linam and Nielsen (1966)

* In September-October only.

where they could not have survived the whole winter. Many of the *An. earlei* were trapped emerging from burrows in April and May, but these may still be considered overwintering sites, since many traps were placed before the mouths of the burrows were free from snow.

The searches in the winter of 1972 - 73 covered the greatest variety of sites. In the winters of 1973 - 74 and 1974 - 75, most of the searching was at the sites that had yielded mosquitoes in the first winter, (rockpiles, burrows and root cellars), thus the results are biassed in their favour.

7.2.1. Rockpiles

Rockpiles created by farmers clearing their fields were quite common in the George Lake area, and will probably increase as new ploughings make further clearings necessary. The smallest rocks were the size of an adult's fist and the largest more than 50 cm across. The piles were mostly at the edges of fields or sloughs. The locations of some of the rockpiles at George Lake are shown on the map (Fig. 66), and one is pictured in Plate 5a. Since the piles sloped gently most of them had a complete snow cover in winter except sometimes for the top on the windward side. Twelve collections were made from 7 rockpiles, 5 at George Lake, (numbers 1, 2, 3, 4 and 6), one at Onoway, 30 km south of George Lake, and one in a ditch by the side of the road 8 km west of Busby and 9 km east of George Lake. Rockpiles 1 - 4 at George Lake were all within 200 m of known breeding sites of *An. earlei* and *Clx. territans*. The rocks were turned and

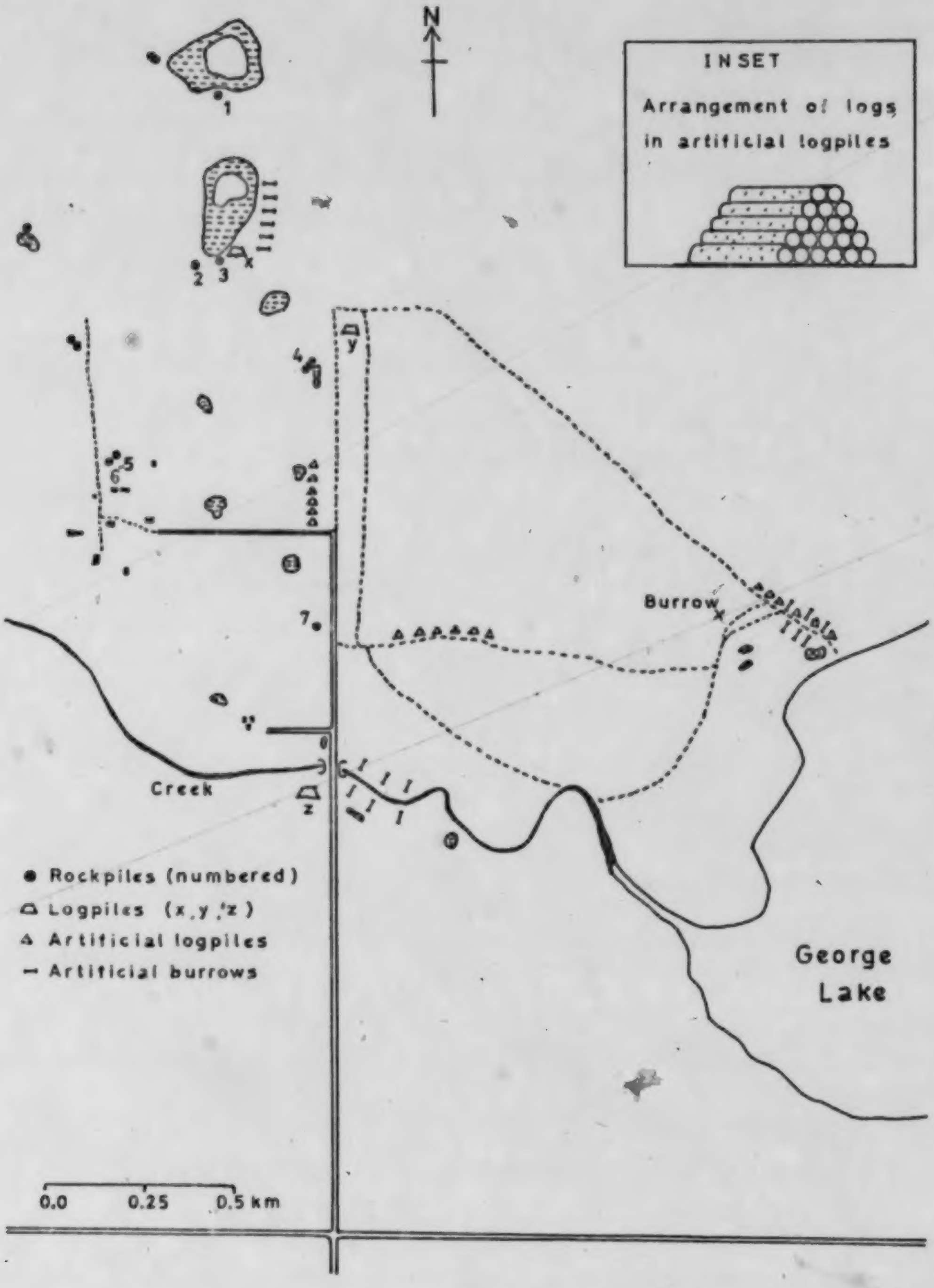
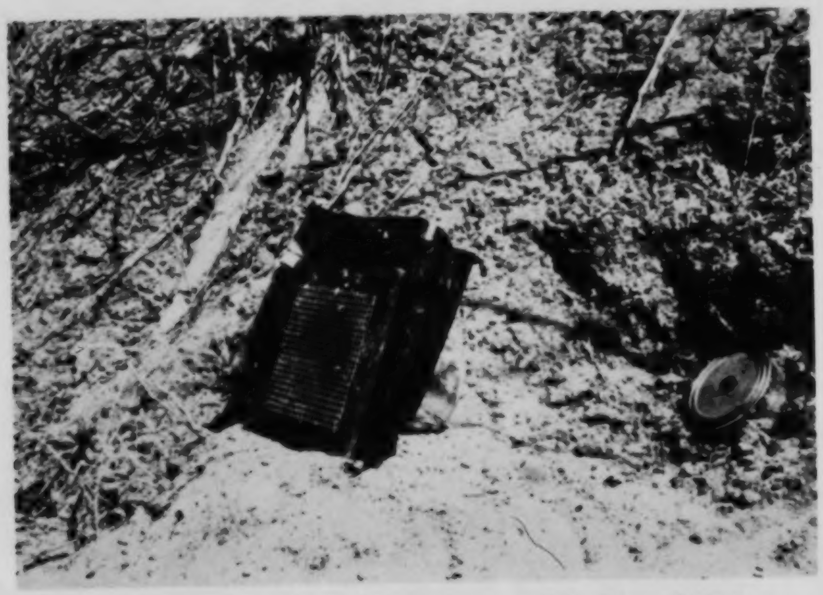

N
INSET
Arrangement of logs in artificial logpiles
1
2
3
x
y
z
4
5
6
7
Burrow
Creek
George Lake
● Rockpiles (numbered)
⌂ Logpiles (x, y, z)
▵ Artificial logpiles
— Artificial burrows
0.0
0.25
0.5 km

Fig. 66. Map of George Lake site showing location of studies on overwintering.

Plate 5

a. Rockpile 6, George Lake, 22/ii/75, with snow cover and rocks in foreground removed.



b. Traps used in badger burrows to catch mosquitoes entering (arranged as on left) or leaving (arranged as in situ on right).

examined individually for mosquitoes, which were aspirated into vials kept on snow until the end of the trip, then stored at +2 C, as were all the overwintering mosquitoes collected. Most of the rocks were dry and easy to handle but a few were stuck together with ice and were freed by tapping lightly with a crowbar. Temperatures within the air and between the rocks were measured with a telethermometer, (Yellow Springs Instruments), the probe attached to a piece of stiff wire and pushed between the rocks. Continuous temperature recordings were made in three of the piles, (Chapter 8).

In the spring of 1974, just before snow melt, rockpile 1 was covered with a black polyethylene sheet fitted with an exit trap at the top, (method of Harwood, 1962). No mosquitoes were caught, perhaps because the plastic was torn by saplings in several places, creating other escape routes.

Since the piles and the rocks varied in size, and some of the searches did not go through a whole pile, the best population estimate was the number per man-hour. This was never more than 4.0, and the rockpiles were a good source of material only because of willing help from staff and students of the University of Alberta.

7.2.2. Logpiles

Fallen trees in the aspen wood and logpiles on the farm created by forest clearing were searched by upturning any stems that could be moved, stripping bark and blowing into crevices. Logpiles were always difficult to search because the branches were tangled together, and almost impossible in winter when some were frozen into the ground.

Mosquitoes were found in three piles (x, y and z in Fig. 66) but only from one pile (y) in winter, and temperatures were recorded in this same pile (Chapter 8). The uneven shape of the piles caused uneven distribution of snow around them, and melting occurred on sunny days where the dark branches protruded. This meant that the space between the logs was connected with the outside air in several places, and got almost as cold.

One logpile was covered with black plastic sheet fitted with an exit trap in the same way as rockpile 1. The plastic was torn in several places by sticks, and no mosquitoes were caught. During August 1974, aspen logs 75 cm long and 10 - 20 cm in diameter were stacked parallel in pyramidal piles of 20, (arrangement shown in Fig. 66, inset). There were 3 rows of 6 piles, two in the aspen wood and one along the east boundary of the farm. No hollow logs were found in the two dominant trees, aspen and spruce.

7.2.3. Root cellars

Root cellars are cool, dark buildings sunk into the ground, used for storing potatoes and other vegetables through the winter. The ones examined ranged in size and complexity from an excavation in a hillside no more than 3 metres wide, roofed with boards and earth, to metal hangers several hundred metres long and one hundred wide with machinery to warm, humidify and circulate the air. It was only in the four smallest that mosquitoes were found in winter. The cellar from which most of the mosquitoes were collected belonged to Mr. J. Walter at Ellerslie, 20 km south of downtown Edmonton. It was

9.1 m long by 6.7 m wide, with walls 2.1 m high and a gabled roof raised a further 1.5 m. the whole structure was sunk about 1.75 m into the ground. The lower floor was divided into four rooms, and separated from the roof space by a ceiling covering about half the area of the building. Two hatches about 60 cm high in the west wall remained open during September, for tipping the potatoes into the cellar, and seemed to be an ideal entry route for the mosquitoes because of their burrow-like appearance. During the winter Mr. Walter tried to keep the temperature at +5 C by means of a small gas heater, and the large quantities of vegetables probably kept the relative humidity high,(means of 71 % on 24/ii/73 and 83 % on 24/iii/73).

7.2.4. Burrows

Most collections were from mammal burrows 15 - 25 cm in diameter and at least 2 m deep, in banks of well-drained sandy soil, and with their entrances usually nearly horizontal. Burrows at four sites were examined:

A: four burrows on north-facing and 9 on south-facing roadside banks 2.4 km east of Dunstable, which is 5.6 km west of George Lake.

A': Eight burrows on a north-facing roadside bank 1.6 km west of Dunstable, were examined only in the fall of 1972, as they were destroyed in 1973 when the road was widened.

B: Six burrows in a west-facing bank on the northern edge of the Sturgeon River valley, about 4 km northeast of St. Albert.

C: Sixteen burrows in the east and west facing banks of a road about 6 km NNW of Dunstable.

According to local people the burrows at all 4 sites were of badgers (*Taxidea taxus*), though a badger was seen only once, on 17/vi/75 at site A. Fresh diggings and footprints were seen at sites A, A', and C but not at B.

A few mosquitoes were collected from the burrow mouths by aspirator, or flushed out by spraying in a household aerosol containing synergised pyrethrins, ("Raid"). Most of the mosquitoes were collected in spring using exit traps based on the design of Harwood and Halfhill (1960). The traps (Plate 5b) were made from cans 10 cm diameter and 15 cm long, from which the bottoms were removed and replaced with a cone of 1 mm mesh black plastic screen pointing inwards with a 1.5 cm hole at the apex. A 2.5 cm hole was cut in the push-in lid and covered with 2 mm mesh metal screen. The can was attached at its base to a 20 cm square of plywood with a hole to receive the can. A skirt of black polyethylene was attached to the edges of the plywood to aid in sealing the trap in the burrow mouth with earth. In the first traps made there was no protection for the entrance cone, and most of them had holes chewed in them, probably by small rodents. Further damage was prevented by a protective screen of 6 mm mesh galvanised wire.

In fall 1972 and 1973, the traps were pointed inwards to catch mosquitoes entering, but none were caught. In spring 1973, 1974 and 1975 the traps were positioned to catch mosquitoes emerging. Once every 7 - 10 days the lids were carefully removed and mosquitoes collected by aspirator: 10 - 20 % escaped. Some traps at site B in 1973 and at site A in 1975 were removed, trampled and shot at by vandals. On 23/x/74 a burrow was discovered in the woods at George Lake, near the top of a gentle slope close to the lake shore. It was at least 1.5 metres deep, with an entrance 15 cm high by 25 cm wide, facing northeast. Three *Anopheles earlei* females were taken resting at the mouth on 23/x/74. A trap was set over the mouth in the spring and summer of 1975, but no more mosquitoes were taken. Temperatures were recorded in the burrow from October 1974 to May 1975 (Chapter 8).

The holes of bank swallows, *Riparia riparia*, are used as summer resting sites by *Cs. alaskaensis* in Alaska (Hopla, 1970). Twelve were examined on 24/ii and 10/iii/73 at Whitemud Creek, just south of 23 Avenue, Edmonton, but no mosquitoes were found. In October 1973, three burrow systems of Richardson's ground squirrel, *Spermophilus richardsonii*, with 13 metres of runs, 5 nest chambers and 6 entrances, and runs of the pocket gopher, *Thomomys talpoides*, totalling 32 metres with 8 entrances, were dug out in pastures at George Lake. No mosquitoes were seen, but some could have been buried in the frequent cave-ins and passed undetected.

In September 1972 eighteen artificial burrows were created by sinking 75 cm lengths of 10 cm (inside diameter) cement drainpipe horizontally into banks at George Lake. They were examined until late October but no mosquitoes were found.

7.2.5. Other sites

Several other sites were searched in winter, including 4 culverts under roads, 1.5 km of storm sewers discharging into the

North Saskatchewan River at Edmonton, a cow barn, pigsties, empty unheated buildings, an abandoned cellar, mats of labrador tea, woodpecker holes, and spaces under loose bark. Some sites in the woods at George Lake examined in late October included piles of dead leaves, tree squirrel runs, 3 mounds of wood ants (*Formica sp.)* and the bases of *Carex* stems in a wet meadow beside the lake, (location C in Fig. 2). On 5/iv/75, when the forest was still deep in snow, eight 1 m^2 pyramidal traps were put out, two in a labrador tea mat, two in a pure aspen stand, two under a spruce tree and two in the *Carex* meadow, to catch mosquitoes emerging from the litter. The traps were examined until late May but no mosquitoes and few other insects were taken.

7.3. Results

Five hundred and eighty-seven unfed females of 4 species were collected from rockpiles, logpiles and root cellars during winter, or trapped emerging from burrows in spring, (Table 53).

7.3.1. Rockpiles

Twelve searches costing 116.5 man-hours revealed 212 mosquitoes, or 1.82 per man-hour, (Table 54). The most abundant species was *Anopheles earlei*, with 108 collected in 10 of 12 searches; the greatest number in one search was 54. Next was *Culex territans*, with 102 in 9 of 12 searches, and a maximum of 28 in one search. The maxima per man-hour were 2.25 *An. earlei* and 4.0 *Clx. territans*. One *Culiseta alaskaensis* was collected at George Lake and one *Culiseta silvestris minnesotae* at Onoway. The mean air temperature (-3 C) was close to the mean temperature between the rocks (-4 C),

Table 53. Female mosquitoes collected from overwintering sites, 1972-75.

	Anopheles earlei	*Culex territans*	*Culiseta alaskaensis*	*Culiseta s. minnesotae*	Total	%
November-March						
Rockpiles	108	102	1	1	212	36.2
Under logs	0	2	0	0	2	0.3
Root cellars	244	1	0	0	245	41.6
March-May						
Badger Burrows(1)	127	1	0	0	128	21.8
TOTAL	479	106	1	1	587	99.9
%	81.6	18.1	0.2	0.2	100.1	

(1) Mosquitoes caught in exit traps.

Table 54. Female mosquitoes collected from rockpiles in the George Lake area, 1972-75.

Date	Pile(1)	Temperatures (C)		Mosquitoes found				Total	Man-hrs(2) searching	No. coll.(2) per man-hr
		Air	Between rocks	*Anopheles earlei*	*Culex territans*	*Culiseta alaskaensis*	*Culiseta s. minnesotae*			
19/xi/72	4	-9	-	0	2	0	0	2	0.5	4.00
27/i/73	1	-11	-	1	0	1	0	2	1.0	2.00
17/ii/73	4	+6	-	0	4	0	0	4	1.0	4.00
15/ii/74	8	+1	-2 to -7(3)	1	0	0	0	1	4.0	0.25
1/iii/74	2	-9	-2	1	8	0	0	9	4.0	2.25
15/iii/74	0	-9	-5	7	3	0	1	11	12.0	0.92
23/iii/74(4)	0	-10	-17	11	3	0	0	14	12.0	1.17
22/iii/74	4	-13	-2	1	3	0	0	4	4.0	1.00
22/ii/75	2	0	-2	4	0	0	0	4	6.0	0.67
22/ii/75	6	+3	-1	17	24	0	0	41	24.0	1.70
15/iii/75	1	+5	-8	11	28	0	0	39	24.0	1.62
22/iii/75	0	-5	-6	54	27	0	0	81	24.0	3.38
TOTAL or MEAN		-3(5)	-4(5)	108	102	1	1	212	116.5	1.82

(1) Numbers refer to piles at George Lake, marked on map in Fig. 66. O = Onoway, B = Busby.

(2) Rough estimates

(3) -7 at 10 cm deep, -2 at 60 cm.

(4) Continuation of pile from which snow removed on 15/iii/74. All mosquitoes found dead.

(5) Means do not include temperatures on 19/xi/72, 27/i/73, 17/ii/73, or 23/iii/74.

but a difference of up to 13 degrees was observed between air and rockpile temperatures on individual days. Air temperatures were higher than average because they were taken in the middle of the day and searching was done on the warmest days possible.

The vertical distribution of *An. earlei* and *Clx. territans* in three of the piles is shown in Fig. 67. Both species were distributed from under the first stone (5 - 10 cm) almost to the bottom of the piles, but the *Clx. territans* were generally higher up. Note that in fitting all the mosquitoes into the plane of the figure, the distance of each one from the surface of the pile has been faithfully represented, but some individuals appear further from the bottom of the pile than they really were. The same data are presented as histograms in Fig. 76.

7.3.2. Logpiles

The only mosquitoes found in a logpile in winter were 2 *Clx. territans* taken on 19/xi/72, thus it was not confirmed that they could survive the coldest month at this site.

7.3.3. Root cellars

An estimated 415 *An. earlei* were seen in 4 root cellars during 3 winters, and 243 of them were collected. One *Clx. territans* was caught in Cernowski's root cellar, St. Albert, on 31/iii/73, but since it was near the door, the weather was warm and snow melt well advanced, it could have flown in from elsewhere the previous day, (Table 55).

Walter's root cellar was estimated to contain 330 *An. earlei* on 17/ix/74 by multiplying the density of approximately 5 per m^2,

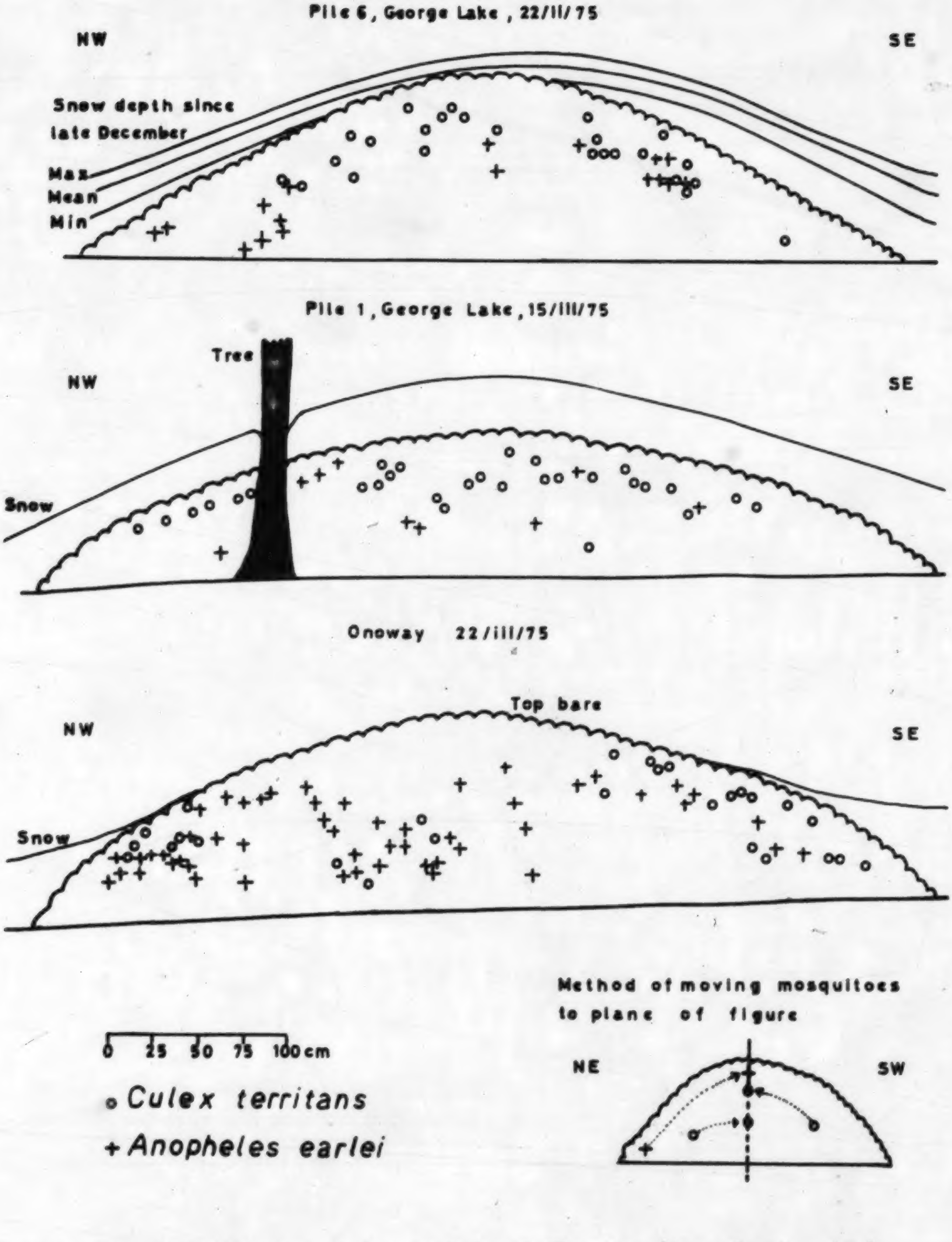

Pile 6, George Lake, 22/II/75
NW
SE
Snow depth since late December
Max
Mean
Min
Pile 1, George Lake, 15/III/75
Tree
NW
SE
Snow
Onoway 22/III/75
Top bare
NW
SE
Snow
0 25 50 75 100 cm
o Culex territans
+ Anopheles earlei
Method of moving mosquitoes to plane of figure
NE
SW

Fig. 67. Distribution of *An. earlei* and *Clx. territans* females in 3 rockpiles.

Table 55. Collections of *Anopheles earlei* from root cellars in fall and winter 1973-76.

Cellar	Date of visit	Temperature (C)			Relative Humidity (%)	Numbers of *An. earlei*	
		Spot	Max(a)	Min(a)		Seen	collected
Cernowski's, St. Albert	24/iii/73	3	-	-	-	2	2
	31/iii/73	6	-	-	-	9	9(b)
	8/ix/73	-	-	-	-	1	1(c)
Kirchner's, George Lake	4/iii/75	-	-	- -	-	4	4
Kuhlman's, Oliver(d)	14/ii/74	-9	-	-	-	12	12
Walter's, Ellerslie	13/i/73	6	-	-	-	47	20
	24/ii/73	9	-	-	71	9	1
	24/iii/73	4	-	-	83	1	1
	5/i/74	6	-	-	-	10	10
	17/ix/74	12	-	-	-	330(e)	12
	16/x/74	10	-	-	-	-	21
	15/xi/74	4	12	3	-	-	24
	16/xii/74	8	9	2	-	-	25
	18/i/75	8	8	2	-	126	62
	14/ii/75	1	14	2	-	-	21
	14/iii/75	4	8	3	-	19	16
	24/ii/76	-	-	-	-	3	3
TOTAL or MEAN		6.2(f)	10.2(g)	2.5(g)	77	415(h)	244

(a) Since previous visit.

(b) Also 1 unfed *Culex territans*.

(c) Also 2 unfed *Culiseta inornata*.

(d) Disused cellar, with one end open.

(e) Rough estimate.

(f) Excluding the open cellar at Kuhlman's, Oliver.

(g) Mean 6.4 C.

(h) Rough estimate of numbers at first visit of each winter.

by the roof area of 66 m^2, since most were on the roof. On 18/i/75 there were 126 left, but 82 had already been collected, thus the survival between September and January was 50.8 %, assuming no further females entered the cellar after 17/ix/74. By 14/iii/75 another 83 had been removed and 19 were seen, thus the survival between January and March was 44.2 %, and the survival between September and March was 11.5 %. On 13/i/73, the first visit, 47 *An. earlei* were seen, and only one was seen on 24/iii/73, after 21 had been removed, thus the survival from January to March was 3.8 %. Ten *An. earlei* were seen on 5/i/74 and 3 on 24/ii/76, the only visits in those winters. These are only rough estimates of resting densities and survival, but it appears that few *An. earlei* would have survived the winter in this cellar. The mosquitoes taken in January, February and March appeared very thin, and the rather high temperatures, (mean 6.4 C) may have caused death from starvation. This cellar also had many spiders, mostly Linyphiids. Remains of *An. earlei* were seen in several of their webs. On 24/ii/76 a Linyphiid was seen feeding on a freshly-killed *An. earlei*.

Ten other root cellars were examined at St. Albert, Oliver, Winterburn and Spruce Grove, but no mosquitoes were found in any of them. The mean temperature in five of them was 4 C, (range 4 - 7), and all were dark and humid. All had doorways big enough to drive a truck through and the mosquitoes may have been unable to get in because the doors were open only for brief periods during the day. Since these cellars were all visited in February and March, mosquitoes could have been present earlier in the winter.

7.3.4. Burrows

In three springs 127 *An. earlei* and one *Culex territans* were trapped emerging from the burrows, in 53 burrow-seasons, or 2.4 *An. earlei* per burrow-season (Table 56). The number per burrow-season was much lower in 1974 (0.5), than in 1973 (7.0) or in 1975 (3.0). Six of the burrows used in spring 1974 had been fitted with entry traps in the fall of 1973, but a mosquito was trapped emerging from each of two of these burrows in the spring, and if the burrows used in fall 1973 are excluded, the number per burrow-season is still only 0.6.

At site B the burrows trapped were the same over the three years, but at sites A and C some old ones caved in, new ones appeared, and trapping was abandoned in a few that were consistently unproductive. No mosquitoes were taken from 49 % of the burrows trapped, and 20.8 % had 1 - 2 in a season, while only 3.8 % had more than 10 in a season, (Table 57). The distribution at sites A and C in 1975 was related to the aspect of the burrow entrances, (Table 58), the greatest numbers being taken from the south-facing burrows, and none from the north-facing burrows at Site A, which were covered with snow about two weeks longer than the other burrows. There were more mosquitoes in burrows with signs of recent mammal activity (fresh digging, pawmarks and displacement of traps) but the association was not statistically significant, (Table 59). Some of the traps, particularly at site A, were disturbed (displaced or bypassed) by humans and other animals. Although some of the mosquitoes collected could have overwintered elsewhere and used the burrows only as a temporary spring resting place, most of those from the disturbed

Table 56. Numbers of unfed *An. earlei* females in exit traps over badger burrows in March, April and May 1973-75.

	Site A George Lake	Site B St. Albert	Site C George Lake	Total	
Numbers of burrows trapped					
1973	0	5	0	5	
1974	8	5	8	21(a)	
1975	8	5	14	27	
Total burrow-seasons	16	15	22	53	
					Number per burrow-season
Numbers of *An. earlei* in traps					
1973	-	35	-	35	7.0
1974	4	5	2	11	0.5(a)
1975	25	3	53(b)	81	3.0
Total *An. earlei*	29	43	57	127	
Number per burrow-season	1.8	2.9	2.6	2.4	

(a) If the 6 burrows that had traps in September and October 1973 are excluded, the number per burrow-season for 1974 becomes 9/15 = 0.6.

(b) 1 gravid *An. earlei* and 1 unfed *Culex territans* also.

Table 57. Distribution of size of catches of *An. earlei* from different burrows, 1973-75.

Year	Number of traps with .... *An. earlei*									Total
	0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	
1973	0	2	1	0	0	0	0	1	1	5
1974	16	3	2	0	0	0	0	0	0	21
1975	10	6	3	3	2	2	1	0	0	27
Total	26	11	6	3	2	2	1	1	1	53
%	49.0	20.8	11.3	5.7	3.8	3.8	1.9	1.9	1.9	100.1

Table 58. *An. earlei* females trapped emerging from burrows with different aspects, George Lake, April-May 1975.

	Aspect				
	North	South	East	West	Total
No. of burrows	4	4	6	8	22
No. with *An. earlei*	0	4	4	6	14
Total *An. earlei*	0	25	17	36	78
Mean *An. earlei*/burrow	0	6.25	2.83	4.50	3.54

Table 59. *An. earlei* females collected from burrows with and without signs of mammal activity, George Lake, April-May 1975.

	With mammal activity	Without mammal activity	Total
Numbers of burrows(1)			
with *An. earlei*	6	8	14
without *An. earlei*	2	6	8
Total	8	14	22
Numbers of *An. earlei*			
Total	33	45	78
Numbers per burrow	4.1	3.2	3.5

(1) For upper part of table, $\text{Chi}^2 = 0.81$, no significant association between *An. earlei* and mammals.

traps were taken before the first disturbance, (Table 60). The single *Clx. territans* was taken quite late in spring, on 15/v/75, but it was from a trap that had never been disturbed. A gravid *An. earlei* was taken at site C on 23/v/75, from a trap that had been displaced some time between 7 and 15/v/75, leaving the burrow mouth open. *An. earlei* had been seen on cattle on 7/v/75, so it seems most likely that this gravid female had taken a blood meal earlier the same spring, and had used the burrow as a temporary resting site. It is not included in the total of overwintering *An. earlei*.

The emergence of *An. earlei* lasted from snow melt until three (1974) to seven (1973) weeks later, (Fig. 68). The interval between the middle of the snowmelt period and 50 % emergence was 21 days in 1973, 5 days in 1974 and 15 days in 1975. In 1973 and 1974, trapping ceased one week after the first occasion that no mosquitoes were taken in the traps. In 1975, trapping was continued from June through August at sites A and C, using 18 burrows and 9 exit traps alternating between visits. This was important because Shemanchuk (1965) found that most of his *Cs. inornata* did not emerge from the burrows until June, while *An. earlei* emerged in April and May. No mosquitoes were taken in the traps at George Lake in June 1975. The numbers taken in July and August are shown in Table 61, combined with the numbers taken in exit traps at site A in August 1974, and the numbers taken by spray and aspirator in 1972 and 1973. Unfed, gravid and male *An. earlei*, one unfed *Cs. inornata* and one unfed *Aedes fitchii* were taken in summer, and unfed *An. earlei* and *Cs. inornata* were taken in fall, all but 4 of the *An. earlei* in early September, the last on 19/ix/74.

Table 60. Numbers of unfed *An. earlei* in disturbed and undisturbed traps over badger burrows, George Lake, April and May, 1975.

	Numbers of *An. earlei* in disturbed traps	in undisturbed traps	Total	%
Before first disturbance	34	34(b)	68	87
After first disturbance	10(a)	-	10	13
Total	44	34	78	100

(a) Plus 1 gravid *An. earlei*.

(b) Plus 1 unfed *Culex territans*.

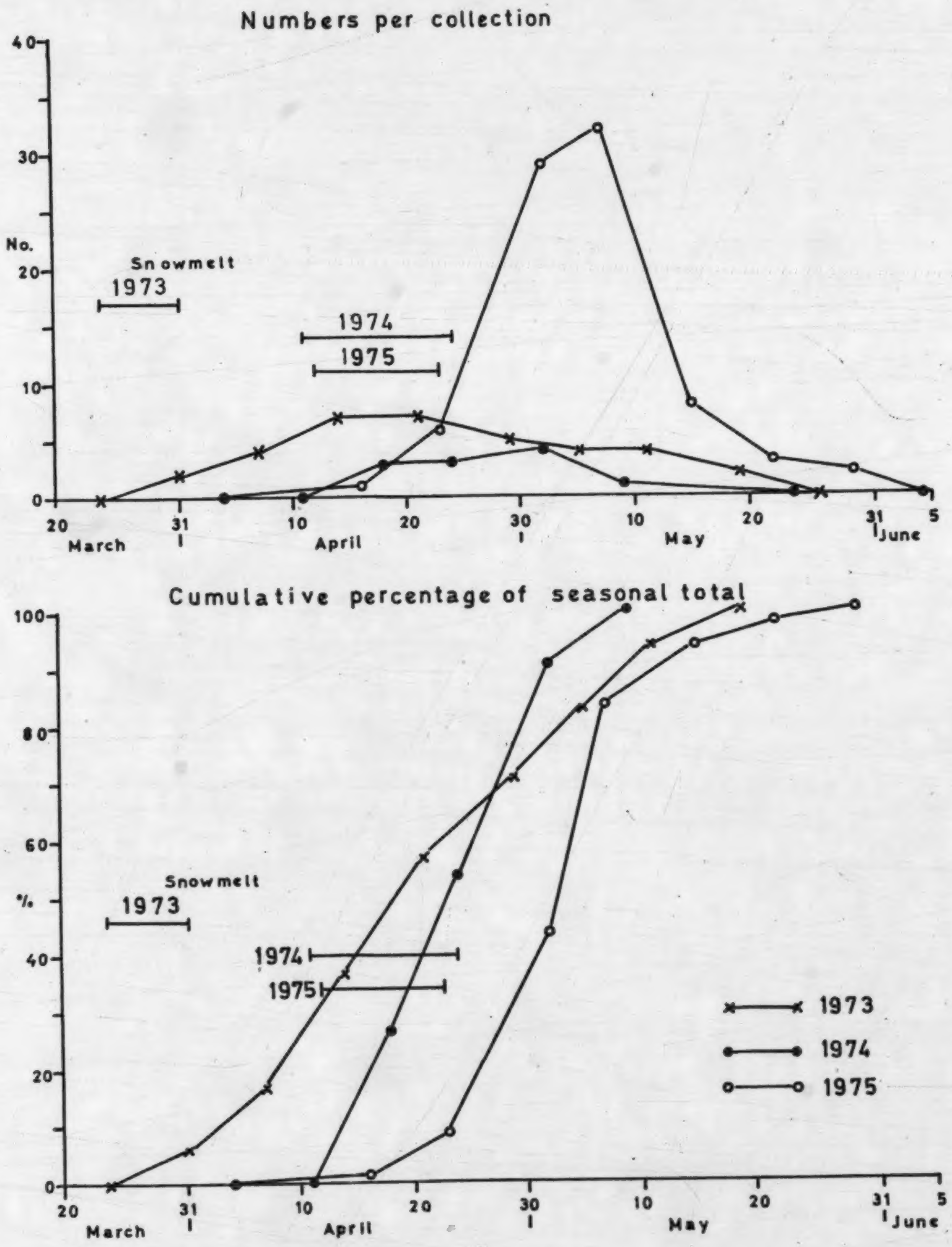


Fig. 68. Dates of emergence of *An. earlei* females from badger burrows in spring, 1973-75.

Table 61. Mosquitoes captured from mammal burrows in Summer and Fall 1972-75. Combined results of exit traps, aspirator and pyrethrum spray collections.

		Anopheles earlei				*Culiseta inornata*	*Aedes fitchii*	Total
		Unfed	Gravid	Males	Total	Unfed	Unfed	
Summer								
July	I	1	2	0	3	0	0	3
	II	0	0	0	0	0	0	0
	III	3	2	4	9	0	0	9
Aug	I	0	1	0	1	0	0	1
	II	0	0	0	0	0	0	0
	III	19	0	1	20	1	1	22
Total		23	5	5	33	1	1	35
Fall								
Sept	I	4	0	0	4	4	0	8
	II	1	0	0	1	0	0	1
	III	0	0	0	0	0	0	0
Oct	I	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0
	III	3	0	0	3	0	0	3
Total		8	0	0	8	4	0	12

7.3.5. Other sites

No mosquitoes were found in any other sites in winter, but some collections in late summer and fall provide clues to possible overwintering sites, (Table 62). *An. earlei* was found in the specially-constructed logpiles until the end of September, in animal sheds until early September, and in the culvert until mid-November. *Culex territans* was taken in the culvert in late August; all collections in fall were from places already shown to be overwintering sites. On 1/x/72 a search of rockpile 1 lasting only 30 minutes revealed 15 female and 2 male *Clx territans* , and 2 female *An. earlei*. The high collection rate, 34 females per man-hour suggest that mortality in the rockpiles may be high, though very few dead ones were found in winter. *Cs. inornata* were taken from the culvert until late October, but it seems unlikely that they could have overwintered there, since the culvert remained open at both ends throughout the winter. In late August 4 *Cs. inornata* and 2 *Cs. alaskaensis* were taken in logpiles. The earliest *Cs. inornata* female was taken on 9/v/73, under a log at the bottom of a pile, next to the leaf litter. She was nulliparous and had a fatbody rating of 2. She could have flown there that same spring, or overwintered there. One female and 9 male *Cs. inornata* were caught in the *Carex* meadow on 30/x/74, but the female had a meagre fatbody reserve, rated at 1, and could have been a late emerger from the breeding site nearby. One *An. earlei* and 2 *Cs. inornata* females were collected from Cernowski's root cellar, St. Albert on 8/ix/73.

Table 62. Captures of mosquitoes resting in possible overwintering sites in fall.

	An. earlei		*Clx. territans*		*Cs. inornata*		*Cs. alaskaensis*		*Cs. s. minnesotae*
	F	M	F	M	F	M	F	M	M
August III									
Culvert	15	8	1	0	5	1	0	0	0
Logpiles	9	3	0	0	4	2	2	0	0
Animal houses + trailer	18	0	0	0	0	0	0	0	0
Rockpiles	1	3	0	0	0	0	0	0	0
September I									
Culvert	3	1	0	0	4	0	0	0	0
Logpiles	2	2	2	0	0	0	0	0	0
Animal houses	6	5	0	0	0	0	0	0	0
September II									
Culvert	1	2	0	0	0	0	0	0	0
Logpiles	6	1	4	1	0	0	0	0	0
September III									
Culvert	0	0	0	0	1	0	0	0	0
Logpiles	2	0	0	0	0	0	0	0	0
October I									
Culvert	3	4	0	0	2	0	0	0	0
Logpiles	0	0	1	1	0	0	0	0	0
Rockpile	2	0	15	1	0	0	0	0	0
Forest floor	0	0	0	1	0	1	0	0	0
October III									
Culvert	0	0	0	0	1	0	0	0	0
Carex meadow	0	1	0	1	2	27	0	1	1
Total	68	30	23	5	19	30	2	1	1

7.3.6. Other animals in overwintering sites

Most sites searched during winter had some living insects and spiders. Fungus gnats (Mycetophilidae) were found at almost all sites, even under the bark of standing trees, which must have been nearly as cold as the air. Adult herald moths (*Scoliopteryx libatrix* L., Noctuidae), were found in the rockpiles, root cellars, burrows and a culvert. Winter crane flies (Trichoceridae) were abundant in several root cellars and were mistaken for mosquitoes by some farmers. Some animals leaving the burrows in spring and entering them in fall are shown in Table 63). Six orders of insects were represented, and there were also spiders, harvestmen (Opilionida) and small toads. The best represented insect families were Mycetophilidae and Gryllacrididae (camel crickets), the latter only at site B. They were not counted on busy days, so the table underestimates their numbers. The Culicidae were the third most abundant family. Although flying insects were well-represented in the spring collections there were very few in the fall collections, which suggests that the protective screens over the traps may have deterred insects trying to fly in. A female *Eucorethra underwoodi* Underwood (Diptera: Chaoboridae) was taken from a burrow at site A on 24/vii/74.

Fewer other arthropods were found in rockpiles than in burrows. Spiders were found mostly near the bottom of the piles; a maximum of 68 was taken from pile 6 on 22/ii/75. Three adult water beetles (Dytiscidae) and 2 water striders (Gerridae) were found in rockpiles close to sloughs. An adult hover fly (Syrphidae) and flea pupae and adults were found in the bank swallow burrows. Searching the 3

Table 63. Arthropods and amphibians trapped from badger burrows in spring and fall.

	Spring (Leaving)	Fall (Entering)	Total	Percent
Number of burrow seasons	54	8		
Insecta				
Orthoptera				
Gryllacrididae	76 +	65	141 +	23.0
Hemiptera				
Gerridae	1	0	1	0.2
Diptera				
Bibionidae	2	0	2	0.3
Culicidae	129	0	129	21.0
Mycetophilidae	124 +	5	129 +	21.0
Psychodidae	1	0	1	0.2
Undetermined[1]	38 +	3	41 +	6.7
Lepidoptera				
Noctuidae	6	0	6	1.0
Undetermined[2]	22	0	22	3.6
Hymenoptera				
Andrenidae	10	0	10	1.6
Formicidae	1	0	1	0.2
Ichneumonidae	1	0	1	0.2
Pompiliidae	21	0	21	3.4
Sphecidae	5	0	5	0.8
Coleoptera				
Carabidae	8	0	8	1.3
Curculionidae	5	0	5	0.8
Elateridae	13	0	13	2.1
Scarabeidae	1	0	1	0.2
Silphidae	3	0	3	0.5
Staphylinidae	0	1	1	0.2
Undetermined	1	0	1	0.2
Arachnida				
Araneida	39	2	41	6.7
Opilionida	0	6	6	1.0
Amphibia				
Bufonidae (Toads)	9	14	23	3.8
Ranidae (Frogs)	1	0	1	0.2
Total	517	96	613	100.2

[1] Mostly Cyclorrhapha.

[2] Probably includes more Noctuidae.

+ More seen but not counted.

anthills revealed 26 adult beetles (8 Staphylinidae, 16 Carabidae and 2 Dytiscidae), 1 moth pupa, 8 fly puparia, 1 centipede and 8 small frogs.

7.3.7. Ovary and fatbody development in overwintering females

The 271 *An. earlei* dissected came in about equal numbers from rockpiles, root cellars and burrows, and nearly all the 87 *Clx. territans* came from rockpiles.

All *Clx. territans* examined were inseminated and nulliparous. Of the *An. earlei* from the burrows one was uninseminated and one was parous. The par was collected on 2/v/74, and would have to have been in the burrow by 24/iv/74 when the trap was set. *An. earlei* were taken at cattle on 23/iv/74, and could have begun biting earlier, but it seems unlikely that this par could have completed an entire gonotrophic cycle that spring.

Most of the *An. earlei* from rockpiles had follicles in stage I, a well-developed fatbody, and the mean F:G ratio was only a little over 2.0 (Table 64). All 10 females with stage IIa follicles in March were from pile 1 on 15/iii/75. The females from the root cellar had mostly stage I follicles throughout the winter, with a few in stage II in January and March. The fatbody ratings showed a decline, and the mean F:G ratios an increase, through the winter months. In about half the *An. earlei* collected from the burrows, follicles had reached stage II, there was little or no visible fat, and the females appeared to be out of diapause. Some of this development may have taken place in the traps.

Table 64. Follicle and fatbody development of *Anopheles earlei* and *Culex territans* females from overwintering sites. All examined were inseminated and nulliparous, except where otherwise indicated.

Species, site and month	Number dissected	Stage			Fatbody rating				Mean F:G ratio	Number measured
		I	IIa	IIb	0	1	2	3		
Anopheles earlei										
Rockpiles										
November	1	0	1	0	0	0	0	1	-	0
February	22	22	0	0	0	3	19	0	2.02	21
March	65	55	10	0	0	7	46	2	2.07	46
Sub-total	88	77	11	0	0	10	65	3	2.04	67
Root cellars										
November	16	16	0	0	0	0	0	16	1.98	16
December	15	15	0	0	0	0	2	13	2.08	15
January	12	8	2	2	0	1	11	0	2.20	12
February	24	24	0	0	0	15	8	1	2.25	11
March	23	20	2	1	0	10	13	0	2.13	10
Sub-total	90	83	4	3	0	26	34	30	2.13	64
Burrows										
March	2	2	0	0	0	0	0	2	-	0
April	33(a)	27	3	3(b)	0	11	20	2	2.16	7
May	58(c)	18	19	21	3	32	21	2	2.46	81
Sub-total	93	47	22	24	3	43	41	6	2.31	88
Total	271	207	37	27	3	79	154	39	2.16(d)	19
Culex territans										
Rockpiles										
November	4	4	0	0	0	0	0	4	-	0
February	26	26	0	0	0	0	18	8	1.66	22
March	55	55	0	0	0	0	43	12	1.44	41
Sub-total	85	85	0	0	0	0	61	24	1.55	53
Root cellar										
March	1	1	0	0	0	0	1	0	-	0
Burrow										
May	1	0	1	0	0	0	1	0	2.5	1
Total	87	86	1	0	0	0	63	24	2.02(d)	54

(a) One uninseminated. (b) One Stage III follicle. (c) One parous.

(d) Mean of means for Sub-totals.

All the *Clx. territans* from the rockpiles had follicles in stage I, most had a well-developed fatbody, and the mean F:G ratio was little more than 1.50. Thus the females appeared to be still in diapause.

7.4. Discussion

The numbers of females recorded reflect more the ease of collection than the relative importance of different overwintering sites to the mosquito population. More *An. earlei* were collected from root cellars than from any other site, but root cellars are far less common than rockpiles and burrows, at least in the George Lake area. Only *An. earlei* were taken in the root cellars, plus one doubtful record for *Clx. territans*, while 4 species were recorded from the rockpiles. Furthermore, it appears that mortality of *An. earlei* in the root cellars is high. Khelevin (1941) noted that the November - April mortality of *An. maculipennis* (subspecies not stated) in the Ivanov oblast, U.S.S.R., was 96.8 % in warm shelters (8 - 10 C) but only 8 % in semi-warm shelters (2 - 6 C). The temperatures and estimated mortality in Walter's root cellar fall between these two groups.

In its overwintering habits *An. earlei* seems similar to *An. m. messeae* in the U.S.S.R., which pass the winter in shelters without mammalian hosts, such as root cellars, or in outside sites such as "depressions round the roots of trees, animal burrows, piles of straw, heaps of brushwood, stacks of peat, reed-grown areas, beneath the bark of trees and many other similar places" (Detinova, 1962, summarising findings of many Soviet workers). *An. m. messeae* overwintered in caves near Archangel, (Timrot, 1941). *An. l. atroparvus* and *An. m. messeae* were both found in cowsheds near Hamburg in summer, but in fall most of the *An. m. messeae* left for outside overwintering sites, while the *An. l. atroparvus* remained in the sheds throughout the winter, and fed on both cattle and fowls (Weyer, 1937). None of the overwintering mosquitoes in the present study showed any sign of blood feeding.

All the overwintering *Clx. territans* and all but one of the *An. earlei* examined were nulliparous. In the U.S.S.R. only nulliparous *An. m. messeae* overwinter, (Detinova, 1962), and in the Sacramento Valley, California, the only *An. freeborni* collected in December and January were nullipars, though some were blood-fed and showing gonotrophic dissociation (McKenna and Washino, 1970). The proportion of parous *Clx. tarsalis* in California reached a minimum between November and January, but they were never completely absent, either in the San Joaquin Valley (Burdick and Kardos, 1963) or in Imperial Valley (Nelson, 1971). In Colorado 2 of 599 collected in a mine in December (0.3 %) were parous, (Blackmore and Dow, 1962).

Neither *An. earlei* nor *Clx. territans* is known to be an epidemic vector of Western Encephalitis. Even if they are involved with virus transmission in nature, the fact that all those collected in winter were nulliparous, and the lack of any evidence for gonotrophic dissociation, make them extremely unlikely winter reservoirs of WE virus. The only known vectors in the area, *Clx. tarsalis* and *Cs. inornata*, were not found in winter. This is reminiscent of the search in Connecticut for the overwintering sites of *Cs. melanura*, a proven vector of Eastern Encephalitis there, which was rewarded with 2,569 mosquitoes of 6 species, but no *Cs. melanura*, (Wallis et al, 1958).

An. earlei in the root cellar had become gonoactive by January, (section 4.3.1.), and those collected from rockpiles in February and March had better developed follicles than the last females taken in fall, but the exact date they became gonoactive was not determined. Nearly all the *Clx. territans* from the rockpiles seemed to be still in diapause, but the appearance of biting *Cs. alaskaensis* and *An. earlei* at snow melt, and of fed and gravid *Clx. territans* shortly afterwards, suggests females of all three species become gonoactive during winter, and do not require reactivation after emergence from their overwintering sites.

It is likely that *Cs. alaskaensis* and *Cs. inornata* overwinter under logs in the forest, close to the leaf litter, where it would be humid in fall and well-insulated in winter. Such sites were difficult to search in winter. The collection of *An. earlei* from a beaver lodge (Section 3.7.) shows how large numbers of females could pass the winter in a small space. Wesenberg-Lund (1921) found very many *Cs. annulata* in a hollow tree in winter in Denmark. In northern Alberta on 4 April, 1792, when there was still more than 25 cm of snow on the ground, Peter Fidler (quoted by Twinn et al, 1948), found live "muskettoes" under the bark of old dry "poplar" trees, in some places in cakes 5 cm thick. These may have been mosquitoes, though in such a microhabitat fungus gnats (Mycetophilidae) cannot be ruled out. Similar mass-hibernation sites of *Cs. alaskaensis* and *Cs. inornata* may exist at George Lake and the fact that no *Cs. inornata* were found does not prove their inability to overwinter there.

8. COLD-HARDINESS

8.1. Introduction

The cold-hardiness of *Anopheles*, *Culex* and *Culiseta* females was studied as a means of delimiting the possible overwintering sites, and as a matter of interest in its own right. Three methods were used:

a) recording temperatures at the females' overwintering sites,
b) measuring the females' supercooling points,
c) determining mortality rates of females at low temperatures.

Since Shemanchuk (1965) recorded temperatures as low as -15 C in a mammal burrow of the type that *Anopheles earlei*, *Culex tarsalis*, and *Culiseta inornata* used to overwinter, it was expected that these species would either have to supercool considerably or be freezing-tolerant, (terminology of Salt, 1961). Since there is no external sign that an adult mosquito is, or has been, frozen it was hoped that comparison of the supercooling points of the mosquitoes with the temperatures in their overwintering sites would provide some indications of freezing tolerance.

8.2. Materials and methods

8.2.1. Recording of temperatures in overwintering sites.

Single-probe, battery-operated temperature recorders (Palmer Instruments Inc., Cincinnati, Ohio) were used at George Lake to make continuous recordings between October and April in a logpile (1972 - 73), three rockpiles (2 in 1973 - 74 and 1 in 1974 - 75) and one mammal burrow (1974 - 75). These recorders had mercury-filled probes, a mechanical recording system, and a 31-day chart driven by a 1.5 volt flashlight battery. Alkaline energizer cells were used and the recorders were enclosed in unheated plywood boxes. One chart drive worked throughout the winter of 1972 - 73, when absolute minima of -36 C and 10-day means of -21 C were recorded at Sion, but in the winter of 1974 - 75 both chart drives stopped on a day when the minimum at Sion was -31 C. Even when the chart drives had stopped, the recorders continued to function as maximum-minimum thermometers. The scales only went down to - 18 C but calibration against a thermocouple at lower temperatures showed that linear extrapolation of the scale to -31 C was as accurate (± 1 C) as in the normal range of the recorder.

From October 1974 to April 1975, hourly temperature records in rockpile 5, George Lake, (location in Fig. 66), were made with a battery operated, 9-channel, thermistor recorder, (Grant Instruments, Cambridge, England). Probes were placed at 10, 20, 50 and 100 cm deep in the pile, and a fifth was suspended vertically 1 m above the apex of the pile, and shielded from the sun by a vaned venturi tube of white-painted aluminum foil, (Unpublished design of Dr. G. Courtin, Laurentian University, Sudbury, Ontario). A six volt alkaline battery was used, and the recording unit was installed in an insulated box with a 40 w pipe heating tape set to +3 C. Some days were missed in November and January due to an electrical fault, and a second recording unit was used for 2 months while the first was repaired. Both temperature ranges on each of the two instruments were calibrated against the thermocouple used to determine supercooling points. Daily maximum and minimum temperatures (as scale units) were transcribed onto punched cards, and the conversion to degrees (including corrections) and calculation of maxima, minima and means for each decade was done by computer, using a Fortran program written by Paul Addison, Department of Botany, University of Alberta.

In none of the series of recordings was it confirmed that the mosquitoes overwintered exactly where the probes were, since doing so would have involved removing the snow cover and making the sites unsuitable for further recordings. Mosquitoes were found in rockpile 1 the winter before and the winter after the temperature recordings were made. The burrow where temperatures were recorded was in the aspen wood, while the burrows from which most of the *An. earlei* were collected were in roadside banks, but no recordings could be made in these burrows because of the danger of vandalism (see Chapter 7). In October 1974, a large rockpile in the farmyard at George Lake was divided into two, (numbered 5 and 6, in Fig. 66). Temperatures in pile 5 and snow cover at 9 points on each pile were recorded until 22/ii/75, when pile 6 was searched and the depths of the 17 *Anopheles earlei* and 24 *Culex territans* found were recorded.

8.2.2. Determination of supercooling points

Supercooling points were determined with the apparatus diagrammed in Fig. 69. The measuring thermocouple, of 0.13 mm (0.005") copper and constantan (copper-nickel alloy) wires, was wedged into a glass tube with a small piece of cork to leave a cell

Mosquito holding cell

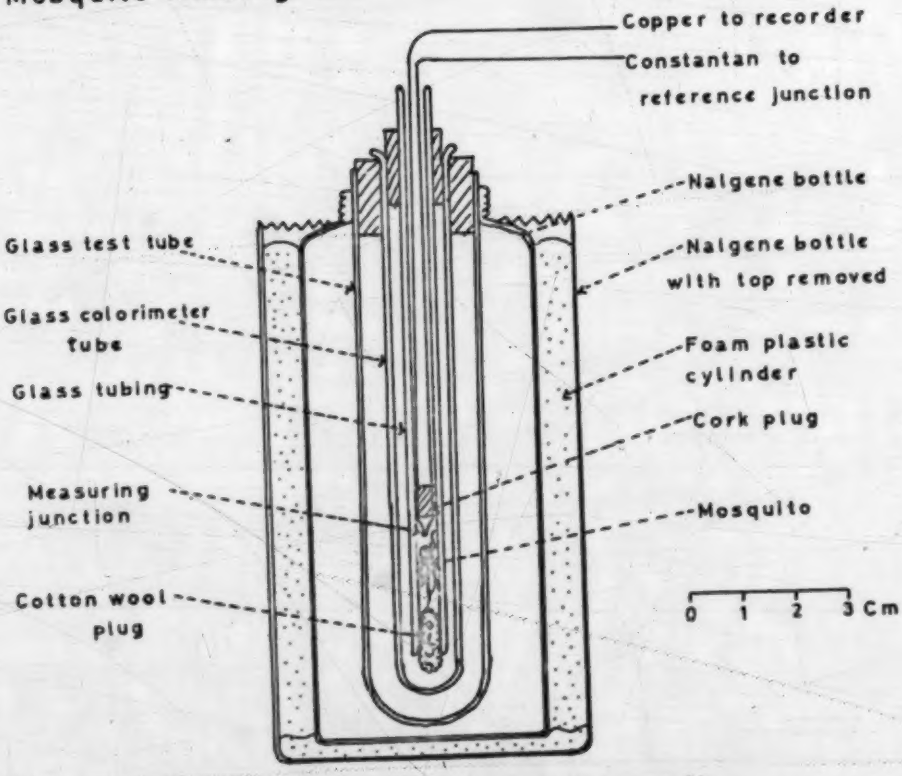

Copper to recorder
Constantan to reference junction
Nalgene bottle
Glass test tube
Nalgene bottle with top removed
Glass colorimeter tube
Foam plastic cylinder
Glass tubing
Cork plug
Measuring junction
Mosquito
Cotton wool plug
0 1 2 3 Cm

Diagram of whole system, not to scale

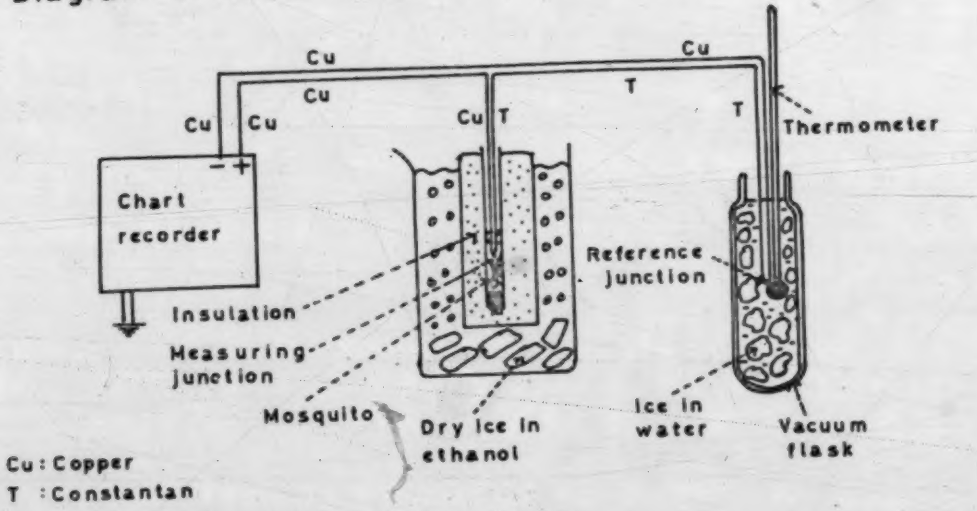

Cu
Cu
Cu
Cu Cu
Cu T
T
T
Thermometer
− +
Chart recorder
Insulation
Measuring junction
Mosquito
Dry ice in ethanol
Reference junction
Ice in water
Vacuum flask
Cu : Copper
T : Constantan

Fig. 69. Apparatus used to measure supercooling points.

25 mm long by 4 mm in diameter in the open end of the tube. Each mosquito was lightly anaesthetized with carbon dioxide and gently pushed head first into the tube until part of its body, usually the mesonotum, touched but was not pierced by the measuring thermocouple. A cotton wool plug behind the mosquito held it against the thermocouple after recovery. The tube containing the thermocouple and mosquito was surrounded with two larger glass tubes, a layer of foam plastic and two nalgene jars; the combined insulation provided a cooling rate of 2 degrees C per minute. The system was cooled in a 500 ml beaker of ethanol and dry ice.

The reference thermocouple, of 0.51 mm (0.02") copper and Constantan wire, was immersed in ice water in a vacuum flask, next to the bulb of a mercury-in-glass thermometer accurate to 0.1 C. The ice bath temperature, (+0.1 to +0.5 C), was added to the (negative) supercooling point measured. The potential differences between the two junctions were recorded on a Chart Recorder (E. H. Sargent and Co., Chicago, Illinois), calibrated against a Honeywell 2725 T/C potentiometer. All leads were enclosed in a grounded wire shield to minimise interference. The millivolt readings were converted to degrees using a curve prepared from data given by the manufacturer of the wires, (Omega Engineering, 1974). The results obtained were accurate to 0.1 C. A specimen cooling curve is shown in Fig. 70.

No attempt has been made to correct for differences in cooling rates since Salt (1969) found that at cooling rates of 0.008 to 16 degrees C/minute, doubling the cooling rate only lowered the mean

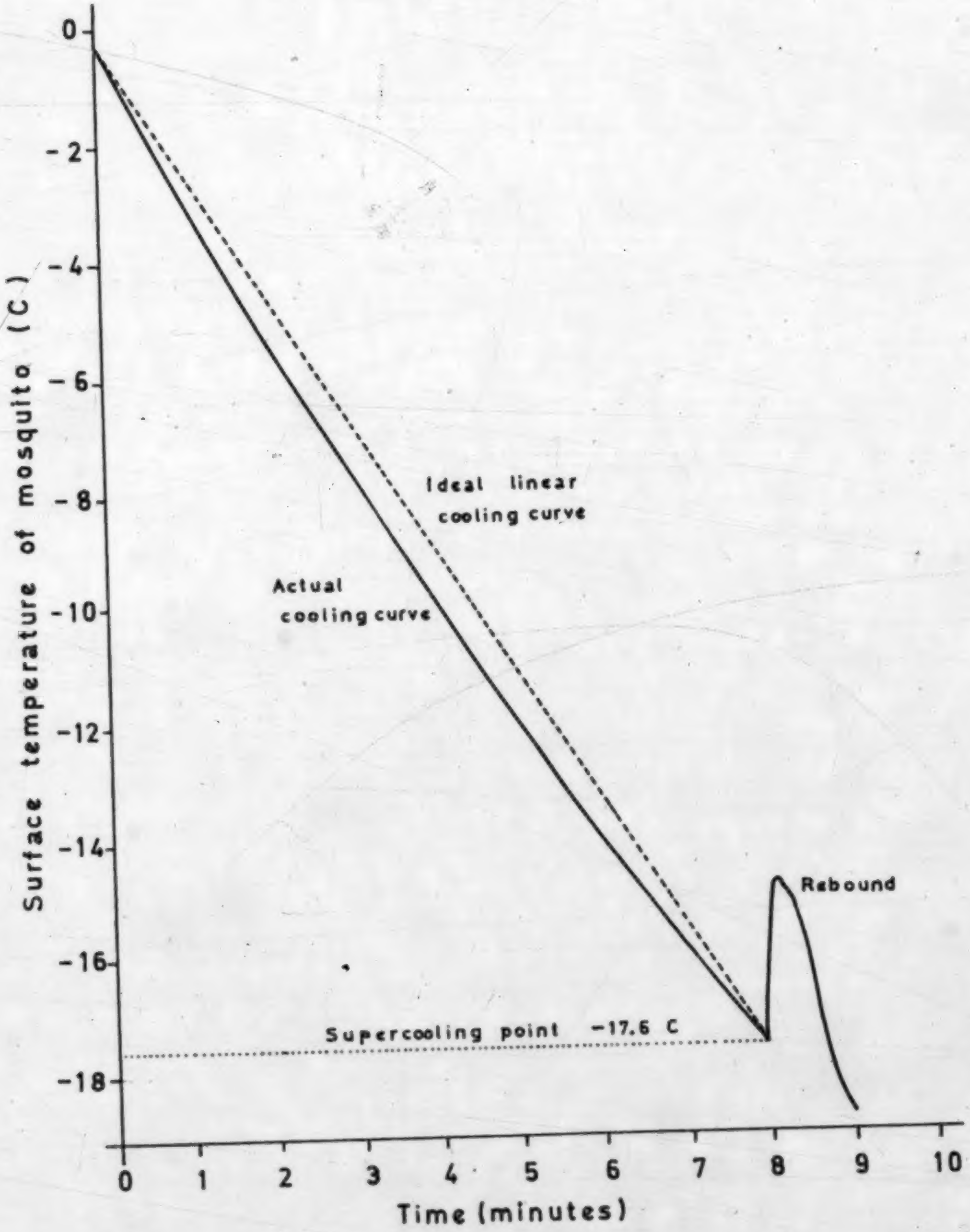


Fig. 70. Specimen supercooling curve of a mosquito.

supercooling point of *Cephus cinctus* Nort. (Hymenoptera) by 0.24 C. All mosquitoes were held at +2 C for at least 24 hours before supercooling point determinations, unless otherwise stated.

8.2.3. Observations of mortality rates at low temperatures

For insects which cannot tolerate freezing the supercooling point represents the lower lethal limit. Above this point the chance of freezing increases with decreasing temperature and increasing time (Salt, 1961), but no simple relationship has been derived between the supercooling point and expected survival times at temperatures above it. The experiments on long-term survival at -5 C give some indications in this area, but what was observed was the number of mosquitoes knocked down from all causes, not simply the number frozen. Other batches of mosquitoes were kept at +2 C under similar conditions, to get an estimate of mortality from causes other than freezing.

Pre-test storage was at +2 C, thus the samples were acclimated to this temperature before the tests began. The mosquitoes in the tests were kept in plastic tubes covered with netting and lined with paper towelling. It was important to line the tubes with an absorbent material, since some early tests had to be discarded when mosquitoes were trapped and died in water drops condensed in the tubes. The tubes were placed on a tray of wet sand, in a plywood box 30 x 45 x 15 cm high, with a plexiglas cover sealed at the edges with masking tape. A wet paper towel was placed over the tubes each time the box was opened, and the presence of ice on the towel the next time the box was opened was a sign that the air in the box was saturated. The plywood boxes were placed in incubators at +2 or -5 ± 1 C. The temperature in a box did not go below 0 C until 2.5 hours after it was transferred from a room at 27.5 C to an incubator at -5 C, and the box and incubator temperature equalised at some time between 5 and 23 hours after the box was introduced. When the box was opened the temperature in the box rose to -2 C and was still at this level 80 minutes later. The experiments with wild-caught *Culiseta inornata* and *Culex territans* began in the fall of 1974, and mortality counts were made on the same group of individuals every five days. The boxes were removed from the incubators, the plastic lids were slid open about 3 cm, and mortality counts made 30 minutes later, by which time the temperatures in the boxes had gone up from -5 to +3 C and from +2 to +12 C. Then the boxes were re-sealed and returned to the incubators.

The median lethal time (LT_{50}) and 95 % confidence limits for each group was obtained using the method of Litchfield and Wilcoxon (1949). In experiments where repeated observations were made on the same individuals, the number of individuals used between 16 and 84 % expected effects (N') was treated as the total number of individuals in the batch. This has the appropriate effect of widening the confidence limits but it decreases the $(chi)^2$ value for the line, and will thus have underestimated heterogeneity in the data.

8.3. Results

8.3.1. Temperature records at overwintering sites

Temperatures in all the overwintering sites during winter were less variable and usually higher than in the outside air. The results are presented as means [(mean daily max. + mean daily min.)/2], absolute maxima and absolute minima for each decade. Since a mosquito's body is small the ambient temperature only has to go briefly below the supercooling point to freeze the mosquito and briefly above the melting point to thaw it. Absolute maxima and minima are therefore important in determining whether freezing or thawing may have occurred, and the time spent frozen. Absolute maxima may also be important in determining the earliest date on which the mosquitoes could have flown from an overwintering site. The mean temperature is more important in relation to rates of diapause determination and depletion of food reserves.

Mean temperatures in the logpile were below 0 C from mid-October to late April, but in the outside air only from mid-October to mid-March, (Fig. 71). The absolute maximum in the logpile was never above 0 C from early November to mid-April, and only reached +1 C in late April. The absolute minimum was -21 C in the logpile (two occasions), and -38 C in the outside air. Snow depths on the logpile were not measured, but the snowfall for the winter of 1972-73 at Sion was 115 cm, less than the normal of 152 cm for 1941-70. There was some snow cover continuously from mid-November to late March, but on the leeward side of the pile there was always a hole in the snow, leading in between the logs.

In the winter of 1973-74, temperature recordings were made in rockpiles 1 and 7, (see Fig. 66), with the probes 20 cm directly below

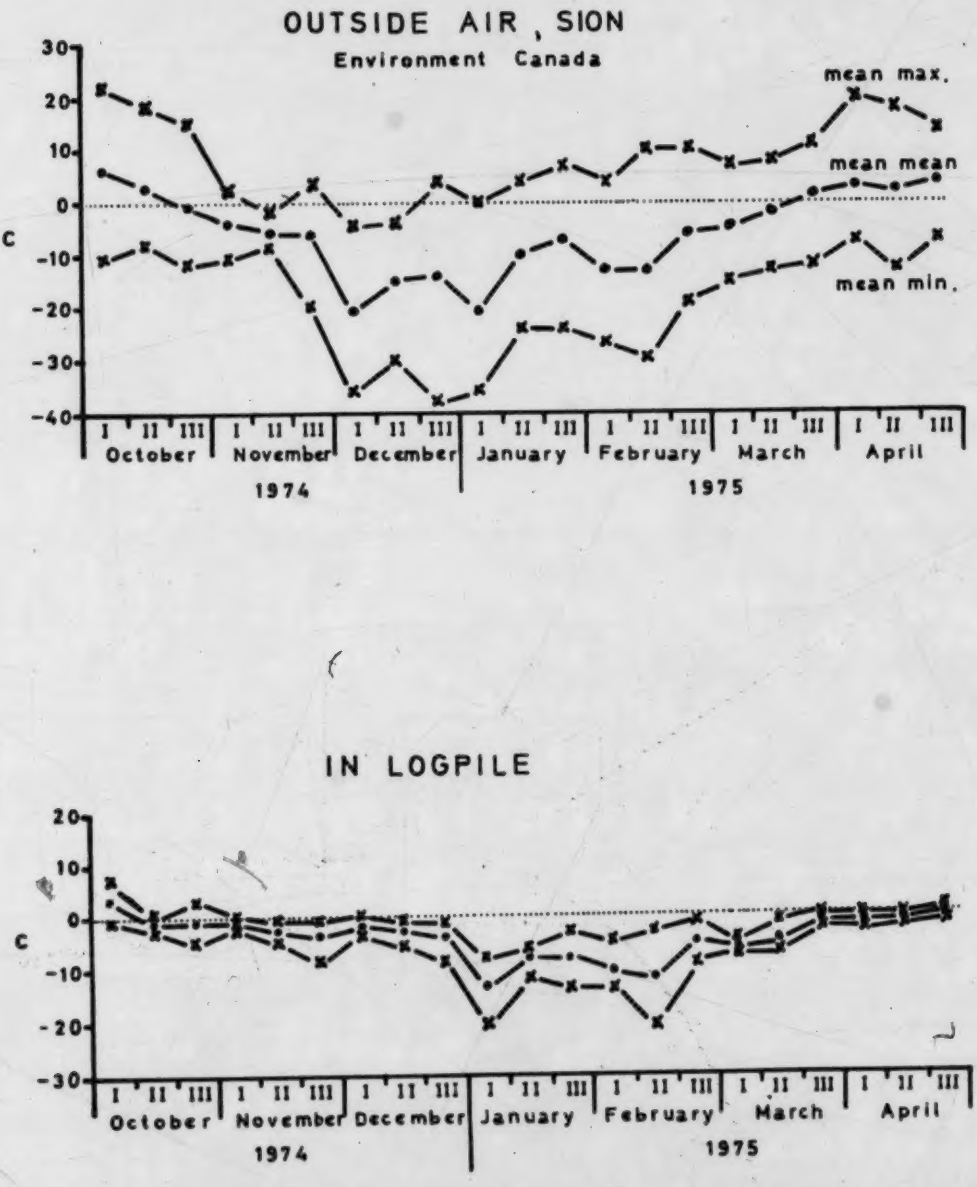


Fig. 71. Temperatures in a logpile at George Lake and in outside air at Sion, October 1972-March 1973.

the apex of each pile. Snow depths at the apices were noted twice or three times monthly. The temperature and snow depth results (Fig. 72) are the means for the two piles. In both the rockpiles the mean temperatures were below 0 C from early November to mid-April, and were between -4 and -8 C for most of the winter, and the absolute minimum was -14 C. The total snowfall at Sion, 196 cm, was higher than normal, and there was a mean of 45 cm of snow on top of the piles in mid-January, the coldest decade. The mosquitoes would have been able to emerge in mid-April 1974 when the snow melted from the tops and an absolute maximum of +4 C was recorded in the rockpiles.

The total snowfall in the winter of 1974-75, 130 cm, was lower than normal. The arrangement of measuring stakes on rockpiles 5 and 6 and the snow cover are shown in Fig. 73. There was no standing snow until 20/xii/74, and although a mean depth of 52 cm was reached at the base of pile 5, the depth at the top of the piles was never more than 14 cm. A few rocks were usually seen protruding from the piles on the windward (NW) sides. Pile 6 had snow cover similar to pile 5 until the day it was searched.

The mean temperature was below 0 C from early November to early April, at 4 depths in rockpile 5 and in the air 1 metre above it, according to the Grant recorder records, (Fig. 74). The bottom of the pile was warmer than the top in mid-winter, but warmed more slowly in the spring. The lowest temperatures recorded in the piles were -18.0 at 10 cm and 20 cm, -17.2 at 50 cm (all in mid-January) and -12.8 at 90 cm (in early February), while in the air -33.6 C was recorded in early February. The hourly records for selected 3-day periods are shown

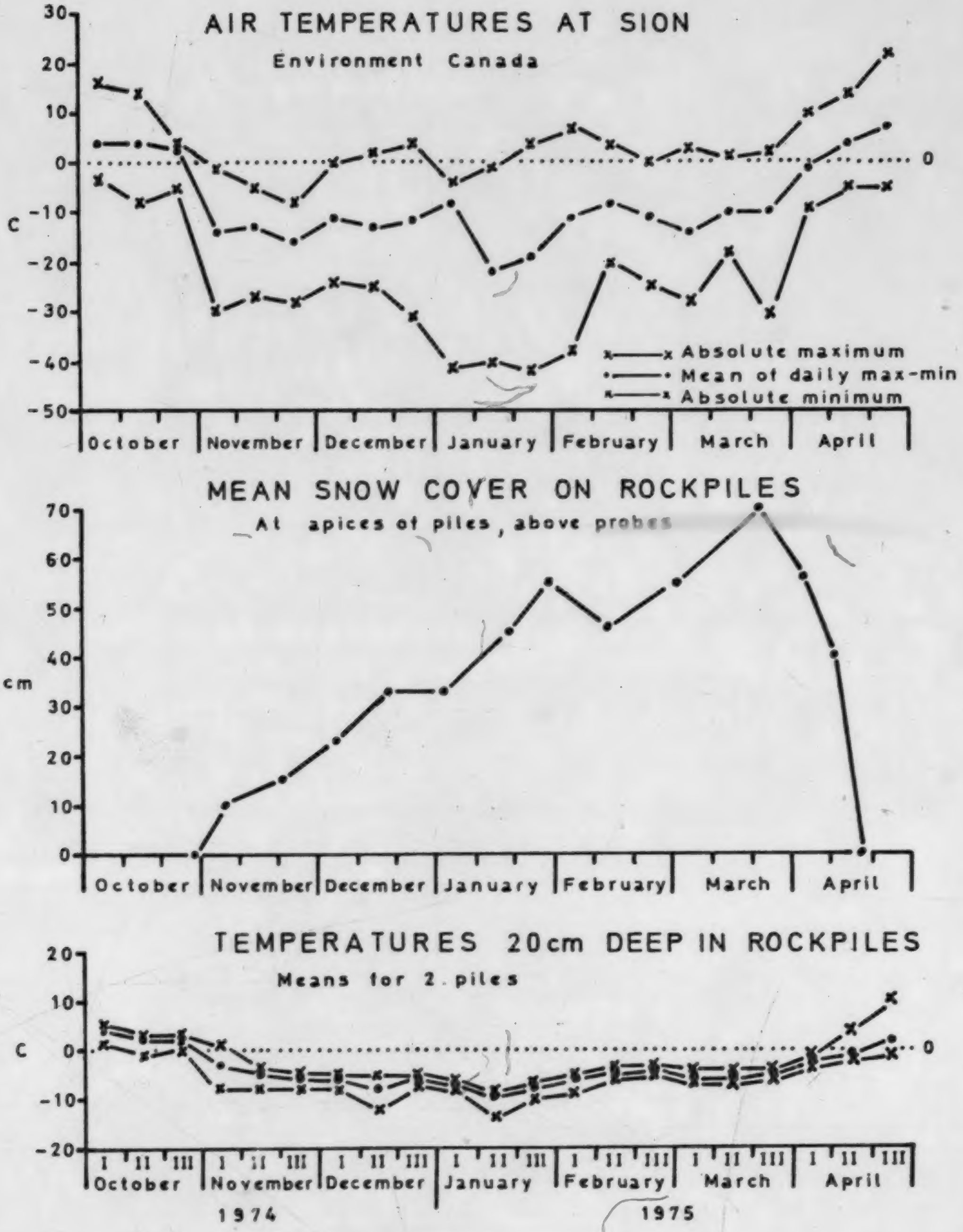


Fig. 72. Mean temperatures and snow cover for two rockpiles at George Lake, and temperatures in outside air at Sion, October 1973–March 1974.

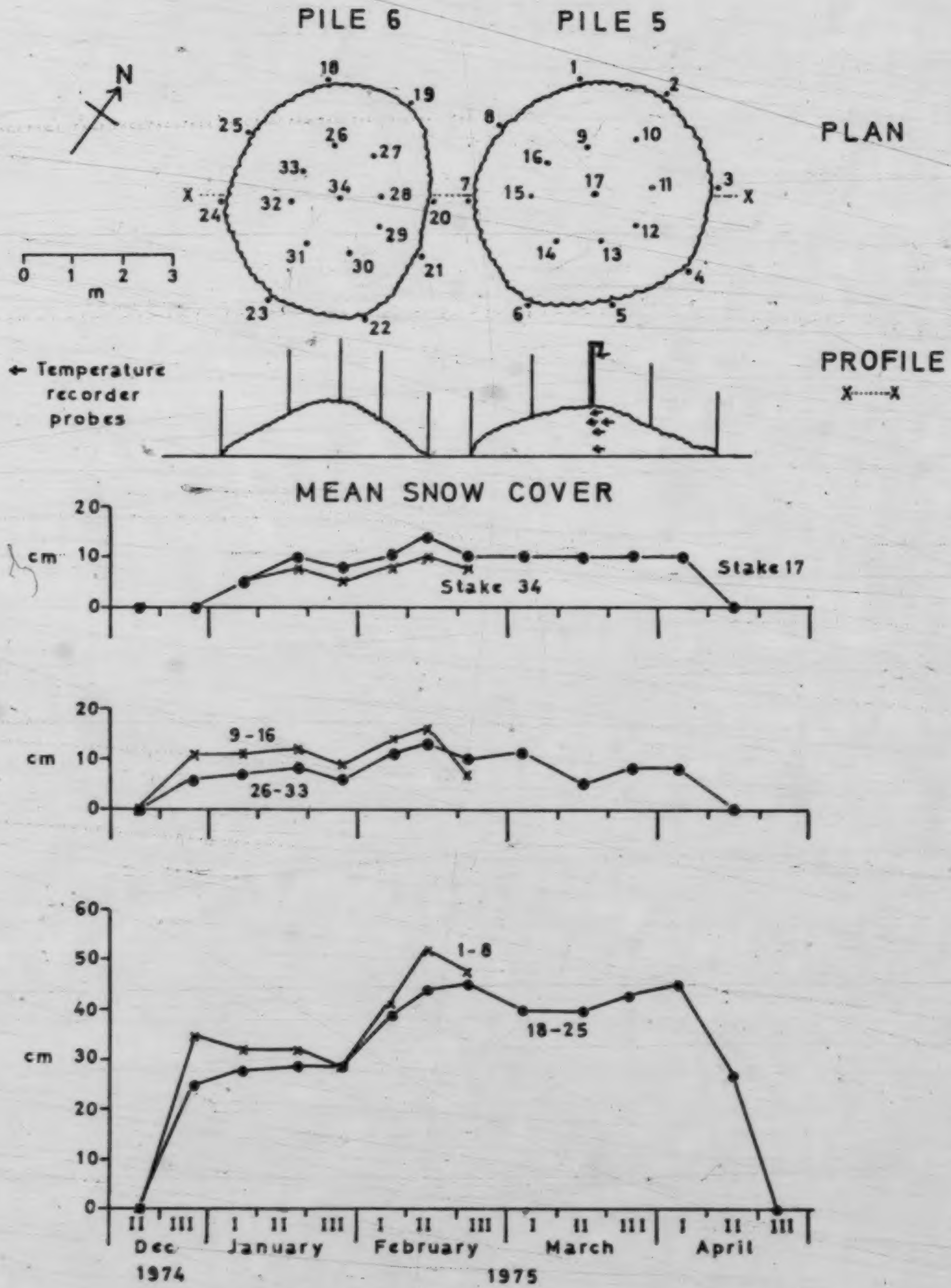


Fig. 73. Plans and profiles of rockpiles 5 and 6, George Lake, and their snow cover from December 1974-March 1975.

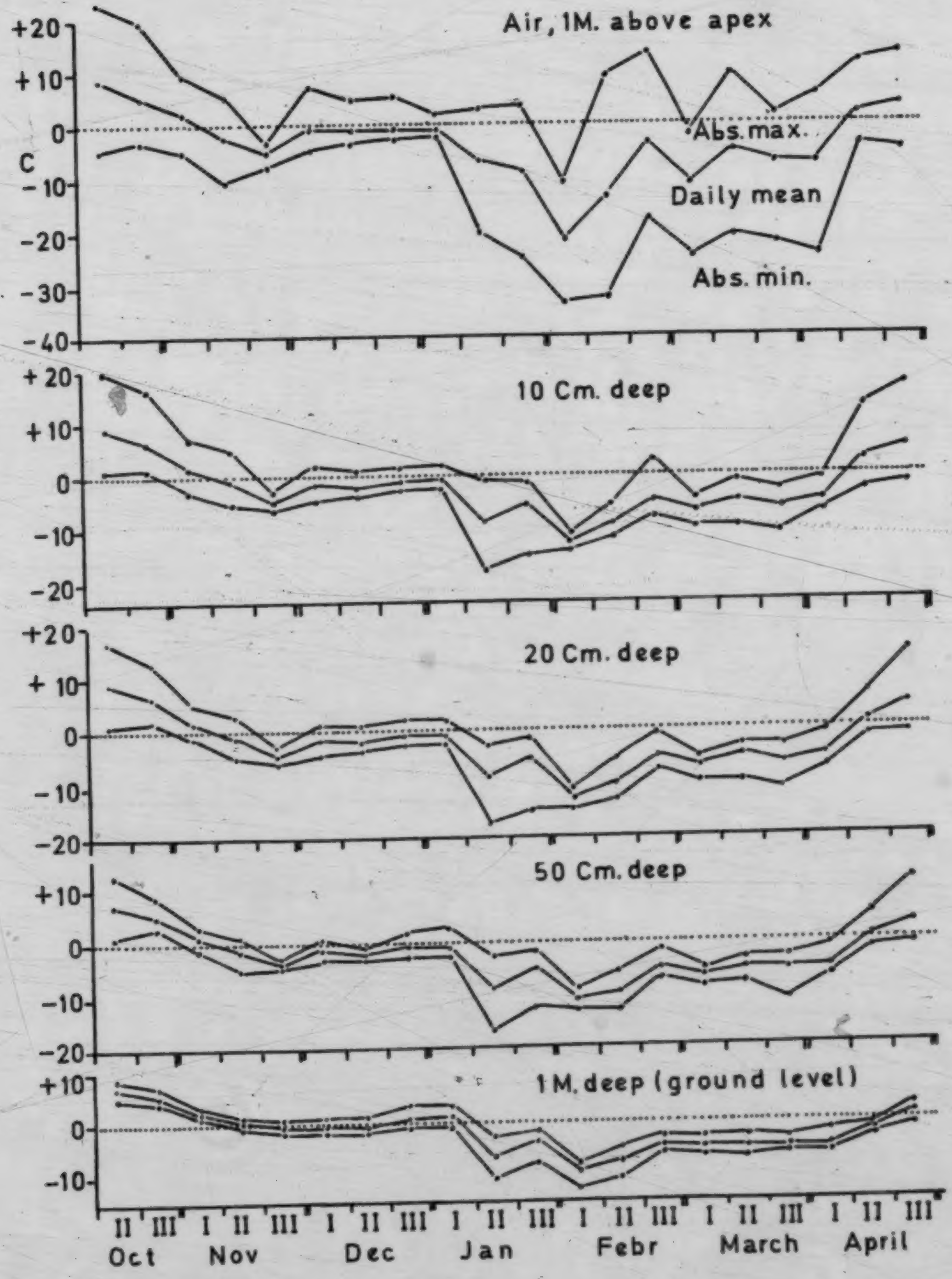


Fig. 74. Temperatures at 5 depths in rockpile 5, George Lake, and in the air 1 meter above it, October 1974-March 1975.

in Fig. 75. Early in the winter (12-14/xi/74), when there was no snow cover, the air temperatures ranged from +5 to -10 C, but 10 cm deep in the pile the range was only +2 to -5 C, and at 90 cm only +1 to 0 C. Late in the winter (10-12/iii/75), when there was 10 cm of snow on top of the pile, the minimum temperatures were -25 C in the air but only -9.5 at 10 cm and -3 at 90 cm deep in the pile. On 12/iii the air temperature rose from -21 to +9 C and the temperature at 10 cm rose from -8.5 to -3.5. Thus a thin snow cover reduced but did not prevent temperature changes in the rockpile following those in the air. In spring (17-19/iv/75) when the snow had melted from the top of the pile, the midday air temperatures reached +12 C but this was surpassed (+14) by the temperatures at 10 cm deep. This was probably a result of insolation of the rocks, and may have caused the mosquitoes to escape earlier from the rockpiles than from the burrows, most of which were still under snow at this time. The temperature at 90 cm deep, however, remained at 0 C throughout the three days.

In three rockpiles examined in February and March 1975, many of the mosquitoes found were less than 20 cm deep and some were even under the topmost stones, 5 - 10 cm deep, (Fig. 76). Thus some of the individuals would have experienced the lowest temperatures recorded anywhere in the piles. The rockpile at Onoway had no snow on the top on 22/iii/75, and only 2 of 54 *An. earlei* and 4 of 27 *Clx. territans* found in it were within 10 cm of the top.

Sion air temperatures for the winter of 1974-75, the Palmer temperature records for 20 cm deep in rockpile 5, and 75 cm inside the burrow are shown in Fig. 77. Of special interest is the

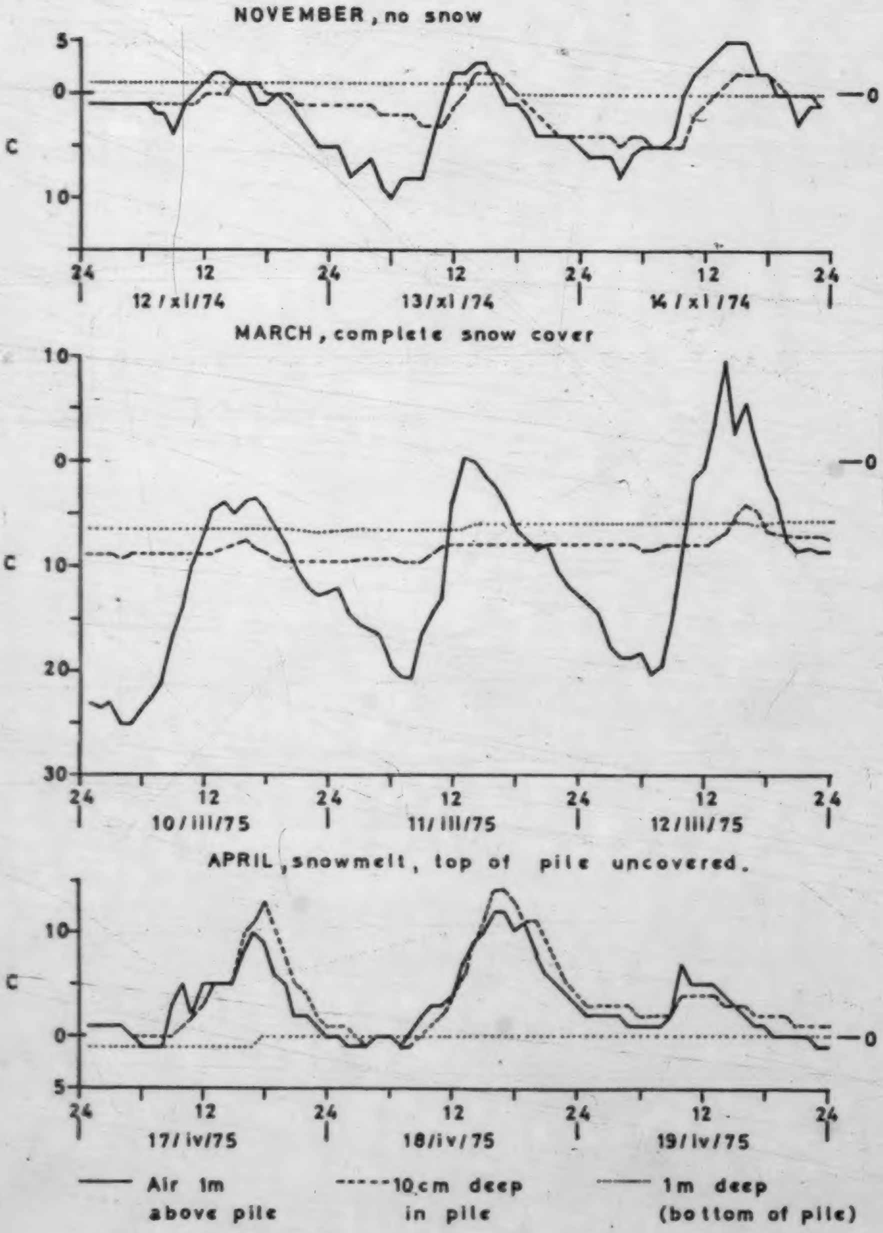


Fig. 75. Hourly temperatures at 10 cm and 1 m deep in rockpile 5, George Lake, and in the air 1 m above it, during 3 selected 3-day periods during the winter of 1974-75.

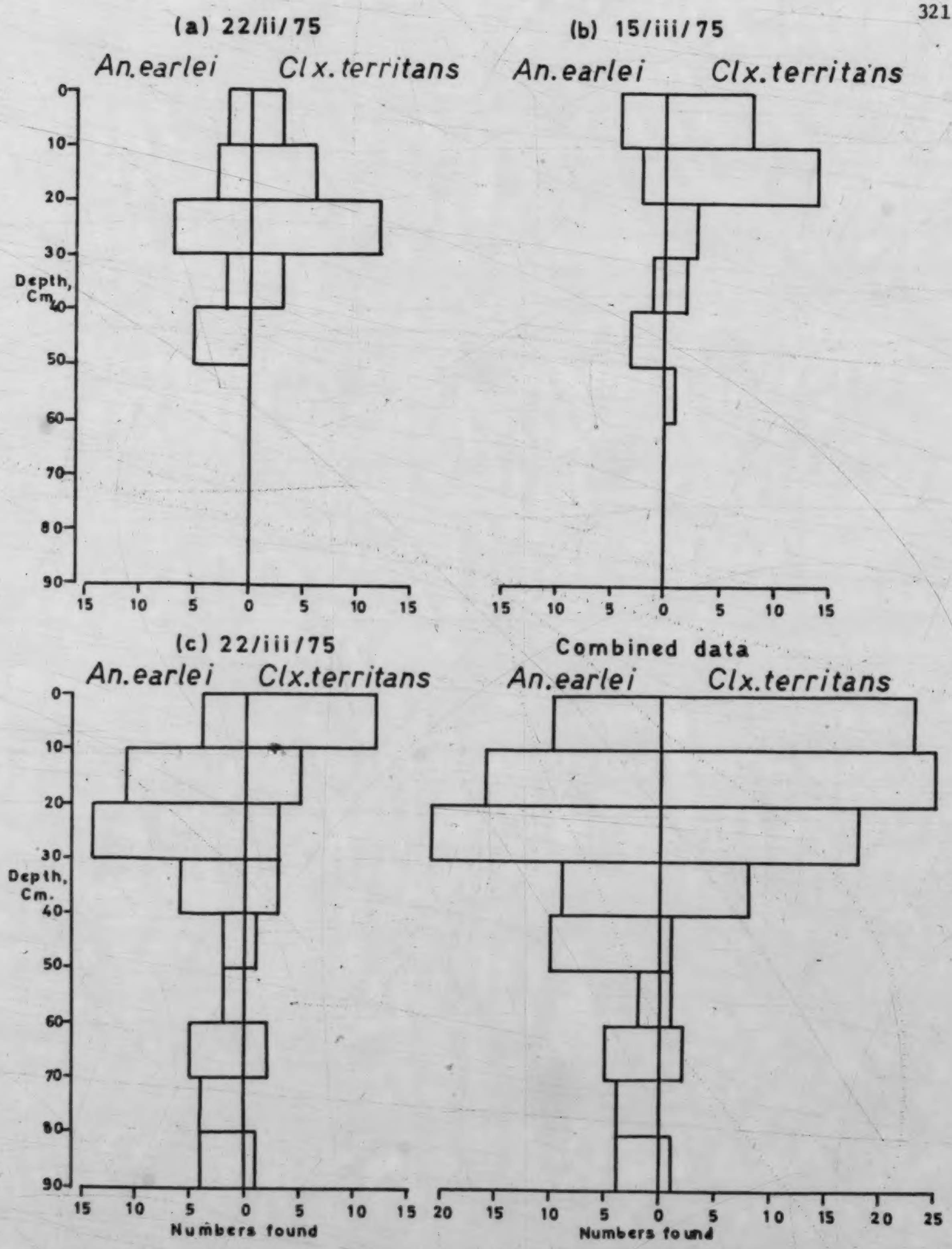


Fig. 76. Vertical distribution of *An. earlei* and *Clx. territans* females in 3 rockpiles, February-March 1975.

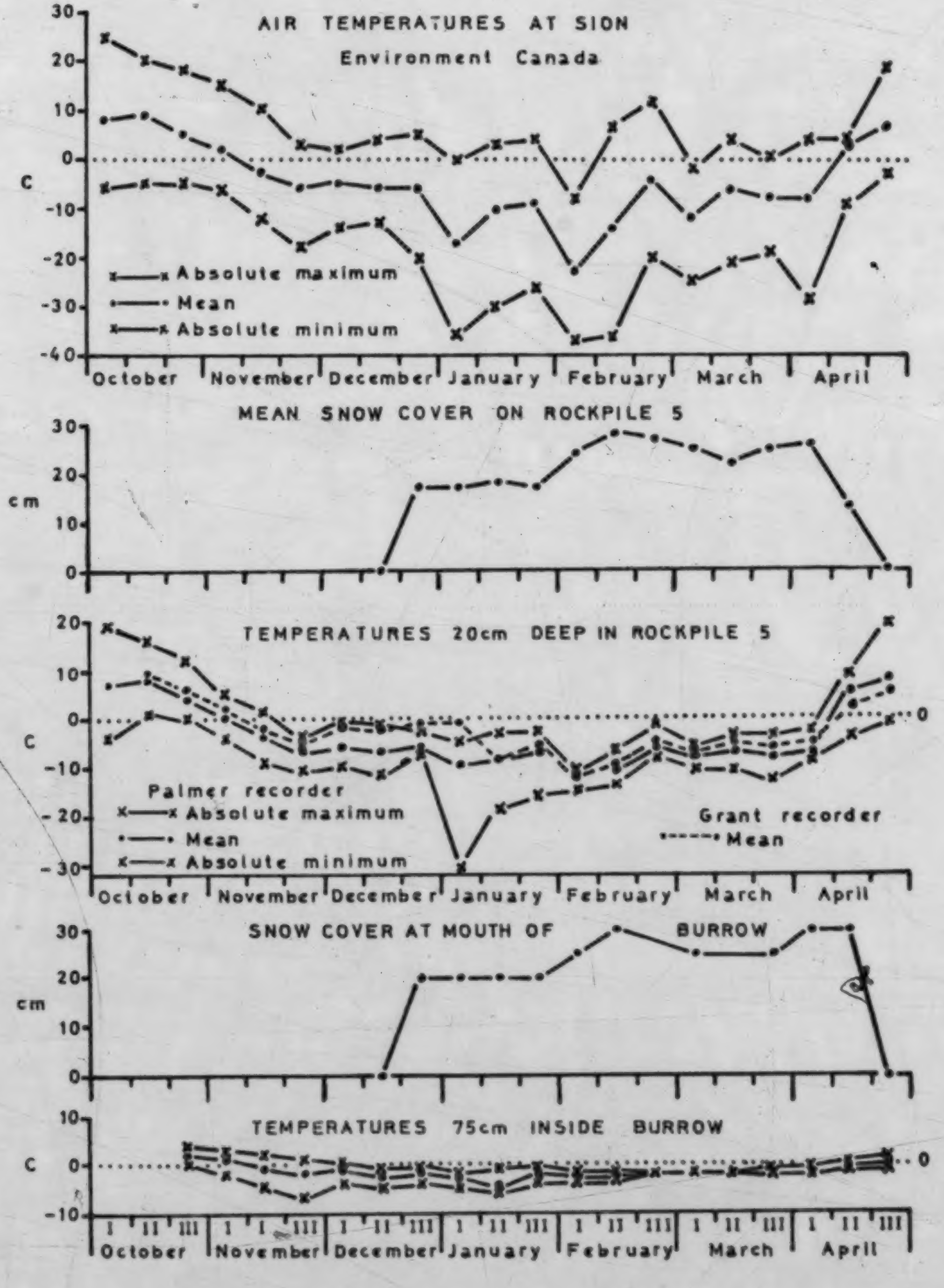


Fig. 77. Temperatures in rockpile 5 and a mammal burrow at George Lake, and in outside air at Sion, October 1974-April 1975.

low of -31 C in early January, which probably occurred on 10/i/75, when the low at Sion was -36 C. This was the lowest temperature recorded in any overwintering site and is lower than the supercooling points of most of the mosquitoes measured. Unfortunately the Grant recorder had been removed for repairs on 7/i and was not replaced until 12/i. Thus we have no information on the minima at greater depths in the pile, and the chart drive on the Palmer had stopped, so we have no information on the duration of the low at 20 cm. The absolute minima for early and mid-February at Sion were -37 and -36 C but the minima for the rockpile in these decades were only -15 and -14 respectively. The extreme low in early January was due to poor snow cover and strong winds. Mean temperatures from the Grant recorder data at 20 cm depth are shown on the same graph. The mean for early January is 9 C higher for the Grant than for the Palmer records, but this is because the Grant recorder was removed before the coldest days in that decade. The mean temperatures from the Grant recorder were 4 - 5 C higher in December and 2 C higher in April than the mean air temperatures at Sion, and the mean temperatures 20 cm deep in the pile, from the Palmer recorder. Since the Grant recorder had developed an intermittent electrical fault by December, the data from the Palmer recorder are more likely to be correct.

The mean temperature in the burrow, (Fig. 77), was between -1 and -5 C from mid-November and mid-April, with an absolute minimum of -7 C in late November, before there was any snow cover. By the end of April the absolute maximum was only +1 C, but the mean of +2 and the maximum of +5 in early May would have allowed mosquitoes to escape. None were trapped emerging from the burrow.

8.3.2. Supercooling points

Seasonal mean supercooling points of 264 wild-caught females of 6 species, are shown in Table 65. *Anopheles earlei* and *Culex territans* were the only species collected in all seasons, and both had their lowest supercooling points in winter, and highest in spring, (overwintered females). *Aedes vexans*, which overwinters in the egg stage, was tested only in summer and had a mean supercooling point of -6.7 C. *Culiseta alaskaensis* and *Cs. inornata* showed decreases from summer to fall. *Culiseta morsitans dyari* was tested only once in late summer and had a mean supercooling point of -7.3 C.

According to unpaired t-tests, diapausing *An. earlei* and *Cs. inornata*, but not *Cs. alaskaensis* in late summer had significantly lower supercooling points ($p < 0.01$) than non-diapausing females, (Table 66). The supercooling points of *An. earlei* from the root cellar, where the temperatures did not go below 0 C were significantly higher ($p < 0.05$) in March than in October, (Table 67), but the supercooling points of females from outside sites (rockpiles and box shelters) were significantly lower in March ($p < 0.01$) than those of females from the root cellar in the same months.

Table 65. Seasonal mean supercooling points (C) of wild-caught female mosquitoes from outside sites, 1974-75.

	Summer (June to August)			Fall (September to October)			Winter (November to March)			Spring (April to May)		
	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$
Anopheles earlei	18	-16.8	0.8	10	-17.0	1.3	30	-23.6	0.5	20	-15.0	1.5
Culex territans	10	-17.3	1.4	16	-20.1	1.1	30	-26.1	1.0	10	-17.2	1.9
Culiseta alaskaensis	32	- 9.7	0.4	9	-10.7	0.8	0	-	-	10	- 5.3	2.3
Culiseta inornata	24	- 9.7	0.5	20	-11.3	0.5	0	-	-	0	-	-
Culiseta m. dyari	10	- 7.3	1.0	0	-	-	0	-	-	0	-	-
Aedes vexans	15	- 6.7	0.5	0	-	-	0	-	-	0	-	-

n = number of individuals

$\bar{x}$ = mean

$S\bar{x}$ = Standard error of the mean

Table 66. Supercooling points of diapausing and non-diapausing mosquitoes, 1974.

Species	Non-diapausing				Diapausing				
	Date	Site	Number	$\bar{x}$	Date	Site	Number	$\bar{x}$	p
Anopheles earlei	15/vii	Boxes	5	-15.3	26-27/viii	Boxes	6	-21.9	< 0.001
Cs. alaskaensis	10/vii	Cattle	5	- 9.1	3-4/viii	Windows	7	- 9.4	n.s.
Cs. inornata	14/viii	Cattle	7	- 8.9	6/ix	Windows	10	-12.1	< 0.05

Table 67. Supercooling points of *Anopheles earlei* females from a root cellar at Ellerslie, and from outdoors at George Lake, during fall and winter, 1974-75.

Month	Root Cellar			Outdoors(1)				
	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$	t	p
September				10	-17.0	1.3		
October	10	-20.2	1.7					
November	10	-17.6	1.8					
December	10	-20.0	0.9					
January	10	-12.6	1.6					
February	10	-15.9	1.6	10	-22.9	1.2	3.5	<0.01
March	6	-15.5	1.2	20	-24.0	0.5	6.5	<0.01

March vs October: $t = 2.3$, $p < 0.05$. March vs. September: $t = 5.0$, $p < 0.01$.

(1) September sample from box shelters, February and March samples from rockpiles.

Factors affecting the supercooling point were investigated in *Cs. inornata* from the Edmonton II colony reared and maintained at 16 hr/20 C (Section 2.14.). All females were supplied with raisins until 1 - 3 days before their supercooling points were determined. At the time of the determinations most females still had syrup-filled crops and well-developed fatbodies. The mean supercooling points of females acclimated at 10 C for 1 or 10 days were significantly lower than those of females kept at 20 C throughout, but the means for females acclimated at +2 C for 1 or 3 days were not, (Table 68). Females fed on my arm 24 hours before measurement had a higher mean supercooling point than unfed females of the same age, but the difference was not significant. The mean supercooling point of the blood-fed females was lower than the supercooling point of human blood *in vitro*, as reported by Lowe et al, (1971). The mean supercooling point of the 40 control females, 5 - 12 days old, was used as a basis of comparison with the tenerals and males. The tenerals, which all had meconium in their midguts when tested at 7 - 19 hours old, had a mean supercooling point significantly lower than the controls. The males had a mean supercooling point higher than the controls, but not significantly so.

Most of the supercooling determinations were terminated immediately after the rebound was observed, but a few cooled further. A few of the mosquitoes vibrated their wings for a while after removal from the chamber, but none recovered enough to stand.

Table 68. Supercooling points in laboratory-reared *Culiseta inornata*. All rearing and holding at 20 ± 2 C except where otherwise stated. n = 10.

	Age	Treated		Control			
Females	(Days)	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$	t	p
Acclimated at 2 C, 1 day	5-7	-18.0	0.6	-16.2	0.6	2.04	n.s.⁽¹⁾
Acclimated at 10 C, 1 day	5-7	-18.6	0.9			2.16	<0.05
Acclimated at 2 C, 3 days	7-8	-17.4	0.9	-18.2	0.3	0.93	n.s.
Acclimated at 10 C, 10 days	11-12	-18.3	0.5	-15.9	0.8	2.40	<0.05
Blood fed, 24 hr before	8-9	-15.6	0.4	-17.2	1.2	1.25	n.s.
Combined controls (to compare tenerals and males)				-16.9	0.4		
Teneral females	7-19 hrs	-19.2	0.9			2.36	<0.05
Males	6-7 days	-14.9	1.3			1.47	n.s.

(1) n.s. = Difference not significant.

8.3.3. Mortality rates at low temperatures

Some *Culiseta inornata* and *Aedes vexans* females collected from cattle during August 1974, were divided into batches and put at +2 or -5 C for various times. All the females were kept in the refrigerator at +2 C for the night after capture, then at room temperature (approx. 20 C) until the evening of the next day, when the tests began, (i.e. approximately 24 hours after capture). Females that had blood visible in their midguts were not used in the tests. The *Cs. inornata* suffered 58 % mortality after 10 days at -5 C but only 4 % after 10 days at +2 C: the survivors soon became active after warming from both temperatures, (Table 69). The *Ae. vexans* died rapidly both at -5 and at +2 C; about half were dead after 12 hours and most of them after 48 hours. When removed from -5 C the *Ae. vexans* were in chill coma and some were still recovering after 5 hours at 20 C; the final mortality count was made after 12 hours. The previous exposure to +2 C could have affected the results for *Aedes vexans*, but a batch of females from the test material showed only 7 % mortality after 60 hours at 20 C.

Culiseta inornata and *Culex territans* females were collected from windows on the U. of A. campus in September 1974, and stored at +4 C for 4 - 30 days during which the mortality was about 5 %. Then they were pooled and redivided into batches which were placed at +2 or -5 C, and observed every 5 days. The *Cs. inornata* at -5 C all died between 10 and 30 days, while at +2 C there was 90 % survival for 30 days and the last did not die until

Table 69. Mortalities of wild-caught *Culiseta inornata* and *Aedes vexans* females after exposures to +2 and -5 C. From cattle, George Lake, August 1974. Stored at +2 C for 12 hr on the day before treatment. Mortality recorded after 12 hr recovery period at 20 C.

Species	Date of Capture	Temperature (C)	Duration of Exposure	Number Exposed	% Mortality
C. inornata	14/viii	-5	10 days	25	68
	14/viii	+2	10 days	25	4
A. vexans	21/viii	-5	12 hr	25	44
	21/viii	-5	24 hr	24	71
	21/viii	-5	48 hr	25	96
	21/viii	+2	12 hr	25	64
	21/viii	+2	24 hr	25	88
	21/viii	+2	48 hr	26	81
	21/viii	+20	60 hr	28	7

120 - 125 days, (Fig. 78). Thus it seems likely that the deaths at -5 C were from freezing. The survival curves for *Clx. territans* at +2 and -5 C were much closer together. At both temperatures 80 % survived up to 90 days and the last individuals died between 220 and 230 days. The mortalities at -5 C do not, therefore, seem to have been due to freezing. The median lethal times and 95 % confidence limits are shown in Table 70. Both species survived longer at +2 than at -5 C, but the data for *Clx. territans* at +2 C were significantly heterogenous, and the 95 % confidence limits overlapped those for -5 C by 54 days.

A comparison was made between the supercooling points and survival at -5 C of *Cs. inornata* and *Cs. alaskaensis* females that had been reared and maintained in the laboratory at 16 hr/20 C. Under these conditions all the *Cs. inornata* would have been gonoactive and all the *Cs. alaskaensis* diapausing, (see Chapter 6). The females were 8 - 10 days old when the exposure tests began, and there were two replications for each species. The *Cs. inornata* in the first and second tests had identical mean supercooling points, (Table 71), the survival data were similar and when combined gave a median lethal time of 9.3 days with narrow confidence limits, (Table 72). The mean supercooling points for *Cs. alaskaensis* were higher than for *Cs. inornata* in both tests but the survival at -5 C was longer. The *Cs. alaskaensis* in the first test had a lower mean supercooling point and longer survival than in the second. The combined data were significantly heterogenous, and the median lethal time of 30.0 days had confidence limits of 16.9 - 53.1

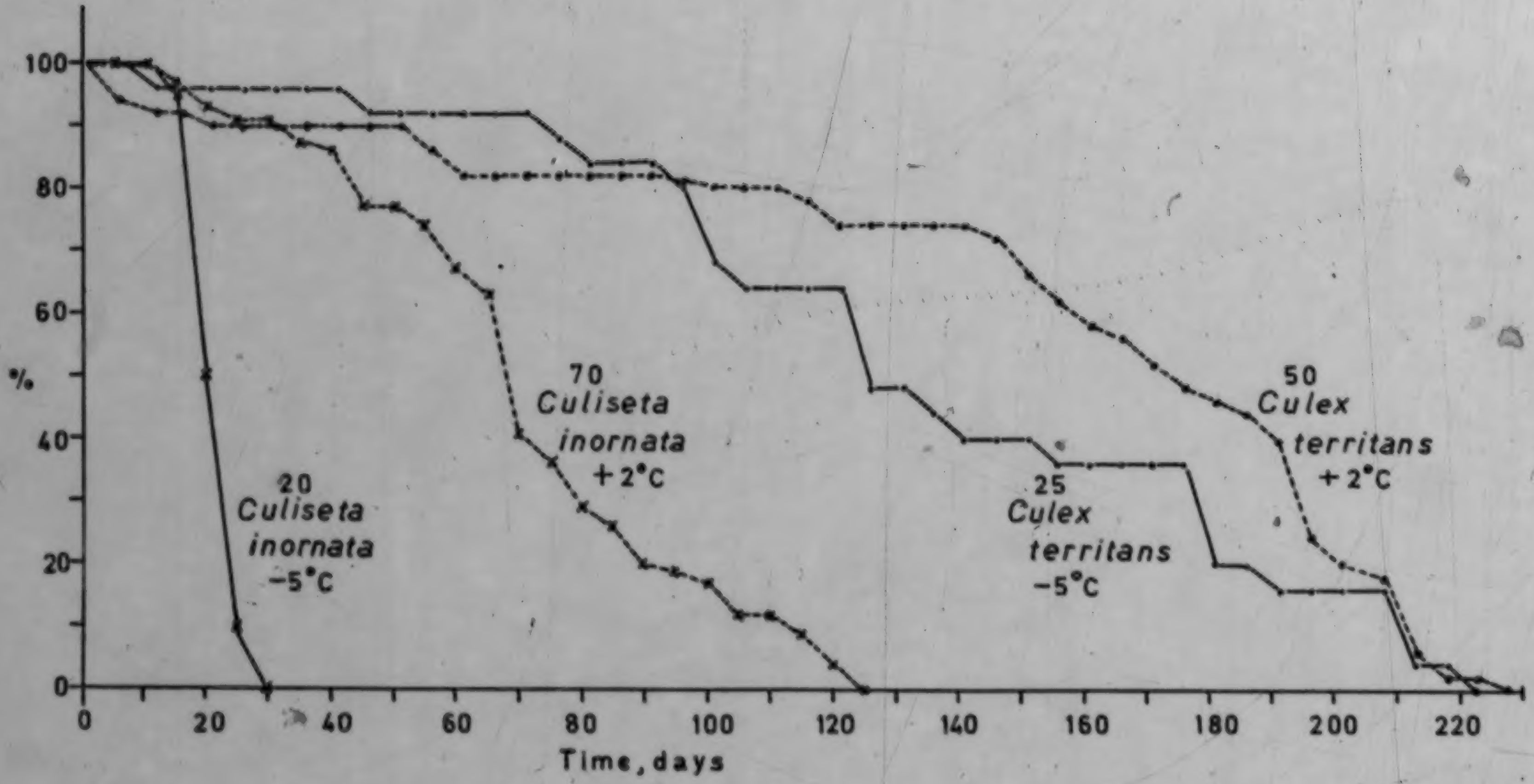


Fig. 78. Survival of wild-caught *Clx. territans* and *Cs. inornata* at temperatures of +2 and -5 C.

Table 70. Median lethal times of wild-caught *Culiseta inornata* and *Culex territans* females at +2 and -5 C. Held at +2 C for 4-30 days before observations began. Warmed every 5 days for mortality counts.

	Culiseta inornata		*Culex territans*	
	+2 C	-5 C	+2 C	-5 C
Number tested	70	20	50	25
Median lethal time (days)	65	20	171	137
95% confidence limits (days)	56-74	18-23	133-220	98-187
Range of survival (days)	1-126	15-30	1-228	7-223
Slope of line	1.49	1.23	1.81	1.37
95% confidence limits	1.34-1.66	1.14-1.32	1.38-2.37	1.35-2.41
$(\mathrm{Chi})^2$ of line	10.332	0.052	90.26*	5.857

* Data significantly heterogeneous at 5%.

Table 71. Supercooling points of laboratory-reared *Cs. inornata* and *Cs. alaskaensis*. Females 7-10 days old, given raisins until 1-3 days before measurement.

	No.	Mean	Range	$S\bar{x}$
Cs. inornata				
Test 1	5	-14.4	-11.8 to -17.1	
Test 2	5	-14.4	-10.5 to -17.9	
Combined	10	-14.4	-10.5 to -17.9	0.7
Cs. alaskaensis				
Test 1	5	-13.5	-9.9 to -15.7	
Test 2	5	-12.4	-11.6 to -13.5	
Combined	10	-12.9	-9.9 to -15.7	0.5

$t = 1.63$, p not significant.

Table 72. Mortalities of laboratory-reared *Cs. inornata* and *Cs. alaskaensis* at -5 C. Females 7-10 days old, given raisins until 1-3 days before tests.

Exposure (days)	Test 1		Test 2		Total	% mortality
	Number exposed	Number dead	Number exposed	Number dead		
Culiseta inornata						
5	10	1	10	1	20	10
8			30	5	30	17
10	10	6	20	9	30	50
12			20	15	20	75
13	10	9			10	90
15	10	10	10	9	20	95
18	10	10			10	100
20	20	20			20	100
Total	70		90		160	

LT$_{50}$ = 9.3 days, 95 % confidence limits = 8.4-10.3. Slope = 1.3513, 95% confidence limits = 1.2489-1.4621. (Chi)^2 of line = 10.68, a good fit.

Exposure (days)	Test 1		Test 2		Total	% mortality
	Number exposed	Number dead	Number exposed	Number dead		
Culiesta alaskaensis						
5	5	0			5	0
10	4	0			4	0
20	4	0			4	0
30	5	0	10	6	15	40
32			20	13	20	65
34			20	16	20	80
36			20	12	20	60
38			10	8	10	80
45			10	9	10	90
50	5	1			5	20
60			5	5	5	100
Total	23		95		118	

LT$_{50}$ = 30.0 days, 95% confidence limits = 16.9-53.1 days. Slope = 1.3162, 95% confidence limits = 1.0347-1.6742. (Chi)^2 of line = 235.43, a poor fit, data significantly heterogenous.

days. These do not, however, overlap the confidence limits for *Cs. inornata*.

8.3.4. Survival of eggs and blood-fed females of *Cs. inornata* at low temperatures.

Two petri dishes of water, each containing 3 egg rafts 2 - 3 days old from the Edmonton I colony, were transferred from the rearing room at 20 C to a freezer at -10 C. Three rafts were removed after 24 hours, and although there was a good hatch from all of them, only 23 of the larvae were still alive 4 days after hatching, and only 8 survived to pupation. The other three rafts were removed after 7 days in the freezer and none hatched. The rafts were broken by the ice crystals, but the individual eggs still floated when the ice had thawed.

Ten 16 - 23 day-old females from the Edmonton I colony were placed 1 hour after engorging on my arm, in a paper-lined plastic tube, which was kept in a refrigerator at +3 C for 24 hours, then transferred to a freezer at -6 C for a further 24 hours. All ten females survived and 7 of them laid eggs.

8.3.5. Observations on the behaviour of females at low temperatures

The lowest temperatures at which females were collected from cattle were 10 C for *An. earlei*, 8 C for *Cs. alaskaensis* and 6 - 7 C for *Culiseta inornata*. On 21/x/72 on the U. of A. campus, when the air temperature was 2.5 C, a *Cs. inornata* female eluded capture by flying off through the falling snow.

Twelve *An. earlei* collected from a root cellar at -9 C, and 3 *An. earlei* and 8 *Clx. territans* collected from a rockpile at -2 C, were kept in paper-lined tubes in a freezer at -7 to -9 C for a few weeks, without apparent harm. They appeared motionless on brief examination but after some hours they had moved up the tube. The tube was inverted on several occasions and each time the mosquitoes regained a head-up posture and moved up the tube. This is the lowest temperature at which any activity has been observed in mosquitoes and possibly in any insects. The Mecopteran *Boreus westwoodi* was active down to its supercooling point of -5.6 C, (Somme and Ostbye, 1969), and the preferred temperature of the Carabid *Pterostichus brevicornis* in January, -5.5 C, was said to be well within the lower limit of mobility, (Baust and Miller, 1970). This lower limit was not stated, but the supercooling point at that season was around -11 C.

Live *An. earlei* and *Clx. territans* were found in the rockpiles with their legs trapped between ice crystals. Some were freed by breathing on the crystals to melt them, others were collected by breaking off the trapped legs, but all survived well for a few days at 0 to +2 C in the laboratory before they were dissected. Two *An. earlei* were also found alive but trapped among ice crystals on the inside wall of Walters' root cellar on 14/11/75; the temperature on the ice patch was -1 C.

8.4. Discussion

There is very little published work on cold-hardiness of adult mosquitoes, and information for species occurring in Alberta is mostly confined to temperature records from overwintering sites. McLeod and McLintock (1947) found 3 living and 3 dead *An. earlei* in a shed in Manitoba where the temperature was -23 C. The authors suspected it could have reached the minimum air temperature of -34 C since there were holes in the roof. A burrow in southern Alberta of the type where *Clx. tarsalis* and *Cs. inornata* spent the winter had a January mean temperature of -2 C and an absolute minimum of -15 C. During collection of *Clx. tarsalis* from rockpiles Trent (1960) in Utah measured temperatures between the rocks of -2 C to +3 C, and Rush (1962) in Oregon recorded -4 to +3 C. When Berg and Lang (1948) collected *Clx. territans* from cellars in Massachusetts, the air and cellar temperatures were the same, so they concluded that the mosquitoes must have experienced the minimum air temperature, which was -22 C. This does not follow. Rockpile temperatures were often the same as air temperatures when collections were made, (Chapter 7), but continuous recordings show that night temperatures in the air were usually lower and the day temperatures higher. Hopla (1965) in Alaska found *Clx. territans* in February in a squirrel nest in a tree, and *Cs. alaskaensis* overwintering in tussocks of grass under the snow; other studies in the area suggested that subnivean temperatures would have been -7 to -9 C. Minar and Hadjkova (1966) in Czechoslovakia found *Cs. alaskaensis* overwintering in cool spaces above ground and moist cool cellars where the temperatures were +0.8 to +5.2 C.

Although none of the *Anopheles*, *Culex* and *Culiseta* studied seemed to be harmed by a month's storage at +2 C, 50 % of *Aedes vexans* females died after 12 hours at +2 C and almost all after 48 hours. In another study all were killed by 24 hours at +4 C, (Costello and Brust, 1971). Night temperatures frequently go this low at George Lake in summer, but *Ae. vexans* is common there. It may be that an exposure of a few hours does no harm, but longer exposures cause death due to metabolic imbalance, as has been observed with honeybees, (Wigglesworth, 1972).

It is surprising that only *An. earlei* was found in the root cellars. *Cs. inornata* females did not survive well at temperatures below 0 C, and might have been expected to find their way into the cellars quite easily, since they often rested on windows.

The lowest reported temperature for adult mosquito survival is for *Anopheles maculipennis* in Siberia, where all survived -18 C for several weeks, 8 % survived 3 hours at -37.5 and all were quickly killed at -40 C. In the same study, 12 % of *Culex pipiens* survived 13 1/2 hours at -15 C, but all died after 4 1/2 hours at -35 C, (Maslow, 1930). Laboratory-reared *Clx. pipiens* were all killed after 4 days at -16 C, (Tate and Vincent, 1936). Both *Clx. pipiens* and *Clx. tarsalis* collected in January in Utah survived better at -2 or 0 C than they did at +3 or +8 C, but this could have been because their food reserves were nearly exhausted, (Mail and McHugh, 1961). In another study (Anderson and Harwood, 1966), *Clx. tarsalis* females caught in summer and fall, or reared in the laboratory, survived better at 0 than at -2 C. From these studies it appears that *Culex* adults will survive better above 0 C than below it, provided their food reserves are adequate and they are not attacked by parasites and predators such as spiders (Whitmyre and Wills, 1970). In several of the studies high humidity seemed to be essential for survival and at temperatures above 0 C, a mosquito would be able to compensate for sub-optimal humidity by drinking. Oda and Kuhlow (1973) demonstrated that *Clx. pipiens* in a cellar had to drink water to survive the winter.

None of the mosquitoes survived chilling at 2 C degrees/ minute down to their supercooling points. But live *An. earlei* and *Clx. territans* were found in rockpile 6 after the temperature had gone below the supercooling points of most of them. It may be that they did survive freezing in the field but not in the laboratory. *Pterostichus brevicornis* (Coleoptera: Carabidae) is freezing-tolerant but nonetheless survived much better if the cooling rate did not exceed 0.3 C degrees/minute, (Miller, 1969). Another freezing-tolerant Carabid, *Pelophila borealis*, froze rapidly when in contact with a drop of water in a freezer, and yet recovered after several days frozen, (Somme, 1974). Another possibility is that the mosquitoes' supercooling points were lower in the field and were raised by holding at +2 C for a few days. Baust and Miller (1972) found that the supercooling point of *Pterostichus brevicornis* was raised at the rate of 0.01 C/hour after transfer from -22 to 0 C, and the glycerol concentration decreased by 0.3 %/hour.

If the mosquitoes did freeze when the temperature went down to -31 C, probably on 10/i/75, they would have remained frozen until the temperature went above the melting point of their body fluids. The earliest recorded date at which this might have been possible is 20/i/75 when the absolute maxima ranged from -1.5 C at 10 cm to -3.1 C at 90 cm. This is based on the assumption that the melting point of the haemolymph was -5 C, a fairly conservative estimate, since the value for overwintering *Bracon cephi* (Hymenoptera: Braconidae) is -17.2 C, (Salt, 1961). Thus the mosquitoes could have been frozen for 10 days.

All the mosquitoes tested supercooled to some extent, even *Aedes vexans* which died rapidly at -5 C, or 1.7 degrees above its mean supercooling point. *Clx. territans* had a lower supercooling point and much better survival at -5 C than *Cs. inornata*. In the experiment with laboratory-reared females, *Cs. alaskaensis* had a higher mean supercooling point than *Cs. inornata*, and yet survived about three times as long at -5 C. Hanec and Beck (1960) found that supercooling points of the larvae of the European Corn Borer, *Pyrausta nubilalis*, were poor indicators of their ability to survive at temperatures below 0 C, but the situation in this species is complicated by the fact that larvae can survive freezing. Smith (1970) found that acclimation at 15 C of adults of the rusty grain beetle, *Cryptolestes ferrugineus*, lowered their supercooling points and increased their survival at subzero temperatures, according to the duration of acclimation. Although it must be admitted that the supercooling points do not give a complete picture of the cold-hardiness of a species, it is of interest that the supercooling points were lowest in mosquitoes overwintering in exposed sites (rockpiles), higher in those overwintering in a cellar above 0 C, and highest in those species which do not overwinter as adults (*Ae. vexans* and *Cs. morsitans dyari*). A strange feature of the results for *Cs. inornata* is that the supercooling points of the laboratory reared females were almost all lower than those of wild-caught females, even of the diapausing females caught in the fall. I can find no explanation for this.

A number of factors including acclimation, diapause, food in the gut, and proximity of ice, have been shown to increase or decrease the cold-hardiness of various insects. Mellanby (1939) showed that in several species of insects the chill coma temperature could be lowered by acclimation for less than 24 hours at moderately low temperatures. Cloudsley-Thompson (1973) demonstrated that short-term acclimation lowered supercooling points of the desert locust *Schistocerca gregaria*, and proposed that the general phenomenon be called the "Mellanby Effect". Some other insect species, however, do not show this effect (Bursell, 1974). *Cs. inornata* reared at 20 C had significantly lower supercooling points after 24 hours at 10 C but not at 2 C. It may be that the processes of acclimation proceed more rapidly at +10 than at +2 C. *Pterostichus brevicornis*, however, seems capable of altering its glycerol content even while frozen, and becomes less cold-hardy at temperatures above 0 C, (Baust and Miller, 1972).

Salt (1971) argued that although insects are cold-hardy during the same season they are in diapause, the association is coincidental. This view is supported by Hanec and Beck (1960) who found that larvae of the European corn borer *Pyrausta nubilalis*, entered diapause in August but did not become cold-hardy until October. Ring (1972) found that diapausing and non-diapausing prepupae of blowflies, *Lucilia sericata*, did not have significantly different supercooling points. On the other hand, Mansingh (1973) found that topical applications of farnesyl methyl ether not only induced diapause in larvae of the wax moth, *Galleria mellonella*, but increased the survival rate and shortened the recovery time after chilling at +4 C. Mansingh presumably would see no contradiction between these varying results because according to his classification of dormancies (1971) a diapausing insect does not acquire all its properties immediately, but in a gradual process of adaptation to the coming winter. Supercooling points were lower in diapausing than in gonoactive females in *An. earlei* and *Cs. inornata*, but not significantly so in *Cs. alaskaensis*. The diapausing and non-diapausing females were not collected at the same time, however. In the tests with laboratory-reared females, the *Cs. alaskaensis* were in diapause and the *Cs. inornata* were not, and the *Cs. alaskaensis* survived much better at -5 C, though their supercooling points were higher.

The presence of food in the gut often raises the supercooling point, and emptying of the gut in phytophagous insects is said to be an essential prerequisite to overwintering, (Salt, 1961). The gut contents are thought to act as nucleators, and leafcutter bees, *Megachile rotundata*, had higher supercooling points after feeding on dusty honey than on clean honey, (Krunic, 1971). Cloudsley-Thompson (1973) found significantly higher supercooling points in blood-fed than in unfed *Rhodnius prolixus*, but I found no significant difference in *Cs. inornata*, and blood-fed females survived for 24 hours at -6 C. Ohyama and Asahina (1972) found two supercooling points in the carpenter ant *Camponotus obscuripes*, an upper non-lethal one representing the freezing of the gut contents and a lower lethal one representing the freezing of the rest of the body. I continued cooling one of the blood-fed *Cs. inornata* to -21.6 C, 5.6 C below the first rebound, but there was no second rebound.

Contact with water often raises the supercooling point due to the increased risk of freezing by inoculation. For example, Danks (1971) noted lower supercooling points in larvae of Chironomids that had been surface dried than in those in contact with free water. The finding of live *An. earlei* and *Clx. territans* with their legs trapped between ice crystals is therefore of interest.

Some Tenebrionid beetles that are freezing-tolerant have supercooling points of -5.5 to -7.7 C, while others that are freezing-susceptible have supercooling points of -12 to -20 C. The freezing-tolerant species have a powerful nucleating agent in their haemolynph, (Zachariassen and Hammel, 1976). It does not seem to be a general rule, however, that freezing-tolerant insects have little supercooling ability. *Bracon cephi*, for example, is freezing-tolerant but supercools to -45 C, (Salt, 1961). If repeated freezing and thawing is harmful, the ability to supercool would reduce the number of times an insect was frozen and thawed during a winter. Another possible advantage of being supercooled is mobility, allowing escape from active, supercooled predators such as spiders.

9. GENERAL DISCUSSION

9.1. Findings in relation to the objectives

9.1.1. Overwintering sites

Females of 4 species were found during winter, all in subnivean or subterranean sites, (Chapter 7). *Anopheles earlei* were taken in badger burrows, but no *Culiseta inornata* were found, though Shemanchuk (1965) took them in burrows as far north as Edmonton. *Cs. inornata* may prefer the burrows of mammals other then badgers. No evidence was obtained that any of the mosquitoes share overwintering sites with vertebrates.

9.1.2. Number of generations per year

Data on seasonal distribution of larvae and adults (Chapter 3) suggest that *Culiseta alaskaensis* has one generation per year, *An. earlei*, *Culex territans* and *Cs. inornata* each have two. *Culiseta morsitans dyari* and *Culiseta silvestris minnesotae* also probably have more than one generation per year. Frohne (1954b) states that *An. earlei*, *Clx. territans* and *Cs. alaskaensis* in Alaska all have one generation per year. In *An. earlei* and *Clx. territans* in Central Alberta, diapause appears in early August, and only the earlier-emerging individuals of the first summer generation will be gonoactive; the rest will diapause. Although the long-term average number of day-degrees at Edmonton would be enough for three generations of *Cs. inornata* per season, the second-generation females would emerge some time in August, and the third generation not until October, (Chapter 5). Selection in the Edmonton region would favour an early diapause, restricting the number of generations to two per year.

McLean (1975) states that in "Arctic America", (defined by him as starting at 53° N), ". . . virus transmission by *Aedes* and *Culiseta* mosquitoes probably occurs infrequently due to their reluctance to imbibe more than one blood meal during their lifetime." (p. 269). If the generalization about feeding habits were true, one would expect that parous females would rarely or never be taken at bait, but pars of both *Cs. alaskaensis* and *Cs. inornata* were taken at cattle. The last *Cs. alaskaensis* were taken in early August, more than three months after the first, and would have been at least triparous. The generalization is probably untrue for *Aedes* also. Graham (1969b) took parous *Ae. excrucians* and *Ae. punctor* in animal-baited traps at George Lake in August, and both are univoltine species, emerging in May and June. In the Ivanovskaya district (57° N) of the U.S.S.R., *Ae. cinereus*, *Ae. cataphylla* and *Ae. punctor* were found that had completed up to 8 gonotrophic cycles, (review by Detinova, 1968). All 3 species are well-represented in "Arctic America". Even at 82° N on Ellesmere Island, some *Ae. impiger* and *Ae. nigripes* females complete 3 gonotrophic cycles, and only the first cycle involves facultative autogeny, (Corbet and Danks, 1973). The probability of local *Culiseta* taking more than one blood meal in their lifetime may be low, and the probability of feeding on humans still lower, but this is not the usual meaning of "reluctance".

9.1.3. Time of onset of diapause

The Follicle:Germarium ratio seemed to be a good indicator of the onset of diapause in the mosquito populations (Chapter 3). Few *An. earlei* and no *Clx. territans* were taken at bait, so in these 2 species the onset of diapause could not be detected by the cessation of blood feeding. The upper limit for F:G ratios of diapausing females was taken as 2.0 for *An. earlei* and *Cs. alaskaensis*, and 1.5 for *Clx. territans* and *Cs. inornata*. These limits agreed fairly well with the dates of cessation of blood feeding, but in all species a few individuals were taken in the fall with F:G ratios above the limits. Recent post-teneral, gonoactive females were not excluded from the diapausing group. In future projects it might be feasible to do so by holding all wild-caught females at natural daylengths and temperatures for a week before dissection, by which time the follicles of the gonoactive females would have grown over the limits, but the follicles of the diapausing females would remain small.

The follicle stages were a less reliable guide to diapause, because females with stage I follicles were taken both at bait and at other sites, (Section 3.16.). Few nullipars had follicles in stage II, however, in fall and winter.

In epidemiological studies it is useful to know not only the abundance of females but also the age composition of the population. Parity rates indicate how many have fed, diapause rates indicate how many are seeking blood meals. The peaks of *Cs. inornata* in light trap and window collections in September were of no epidemiological importance, since they were composed of diapausing individuals.

9.1.4. Blood and sugar feeding habits

Blood meal identifications and bait catches suggested that cattle were the usual hosts for *Cs. alaskaensis* and *Cs. inornata*, and birds the usual hosts for *Cs. m. dyari*. It was not discovered where and on what hosts the overwintered *Cs. inornata* females take their first blood meal, if the first gonotrophic cycle is anautogenous. The blood feeding habits of *An. earlei* were not clarified. A few females were taken at cattle and humans, blood meal identifications indicated that most had fed on rabbits, though these hosts were uncommon, and one collection made at the end of the study (Section 3.7.) indicate that beavers may be preferred hosts. Blood meal identifications of female mosquitoes from Edmonton indicated that *Cs. alaskaensis* and *Cs. inornata* flew at least 1.6 km after blood feeding, and some did so within one day of the meal, (Section 3.4.).

Females and males of most of the species were seen on flowers, most often of *Tanacetum* and *Solidago*, (Section 3.1.4.). The females included diapausing, non-diapausing, fed and gravid individuals. Many of the females dissected had syrup in their crops, but it was not established that this was derived entirely from flowers. Chironomid midges have been observed feeding from honeydew deposits on leaves by Downes (1974), who suggests that this habit may be a basic feature of the Diptera. Such honeydew deposits are common in the study areas in fall and may be an important source of winter food reserve for mosquitoes, especially those that emerge after the flowers are over. Flowering of most of the known host plants was over by the end of September, (Section 3.1.4.).

9.1.5. Seasonal fatbody development

Females of all species except *Cs. m. dyari* appeared to be contain the most fat in the fall, but diapausing and non-diapausing females could not be distinguished by fatbody ratings alone, as there was too much overlap between them.

The proportions of *An. earlei* and *Clx. territans* with abdomens distended with fatbody increased rapidly in late summer, and in fall females of these two species were more consistently fat than those of *Cs. inornata* and *Cs. alaskaensis*. Only 12 % of the *An. earlei* and 3 % of the *Clx. territans* had syrup in their crops, compared with 27 % of the *Cs. alaskaensis* and 63 % of the *Cs. inornata*, (nullipars at sites other than bait). It may be that the first two species convert sugars to fats more rapidly. As the fat reserve is exhausted more rapidly at higher temperatures, the fattest females can be the most versatile in their overwintering habits. Even though *An. earlei* seemed to have the best-developed fatbodies, very few survived the winter in a root cellar at a mean temperature of 6.4 C, (Section 7.3.3.). *Cs. inornata* may enter such sites, but their chances of overwintering there seem even less, if their metabolic rates are the same as those of *An. earlei*.

9.1.6. Blood feeding and assimilation in diapausing females

Although a few females in the first diapause induction experiment displayed gonotrophic dissociation, (Section 6.1.2.), there was no evidence of this phenomenon in any of the species in nature. This contradicts Shemanchuk and Morgante's finding (1966) of gonotrophic dissociation in *Cs. inornata* in August in southern Alberta. Most diapausing *Cs. inornata* collected in Edmonton in September fed on human blood at the first exposure, but there was no trypsin activity in the midgut and most of the blood was expelled earlier than in laboratory-reared, non-diapausing females, (Section 4.3.4.).

The knowledge that digestion of blood does not occur in experimentally-fed, diapausing *Cs. inornata*, but does occur in diapausing mosquitoes that show gonotrophic dissociation, may give some clues to the control of blood digestion. In species that show gonotrophic dissociation, feeding and digestion occur but ovarian development is inhibited; in experimentally-fed *Cs. inornata* digestion is inhibited, and in many species including *Cs. inornata*, *An. earlei*, *Clx. territans* and *Cs. alaskaensis*, feeding is inhibited in nature. The fact that inhibition can occur at different stages suggests that they are not all controlled by the same mechanism.

Gillett, Cole and Reeves (1975) found that in *Aedes aegypti* small blood meals were expelled prematurely from the gut and there was no egg development. Spielman and Wong (1974) also demonstrated that meals of less than 1 mg whole blood did not stimulate egg production in *Ae. aegypti*, but the volume of the meal was not the only critical factor, since a small meal supplemented by saline enema up to normal meal size resulted in egg production, and saline alone did not. Thus the quality as well as the quantity of fluid in the gut is important. Bellamy and Bracken (1971) found that in *Culex pipiens* the number of eggs that matured was related to the amount of protein in the meal. Since development of the appropriate number of follicles began soon after the meal, the authors concluded that the mosquitoes must have a means of assessing the nutrient content of the meal before its complete digestion. A secretogogue mechanism for controlling proteinase levels depends on quantitative determination by the secretory cells of the protein content of the meal. The diapausing *Cs. inornata* took smaller blood meals than the non-diapausing ones, and expelled them earlier from their guts, thus it may be that the diapausing females did not digest their meals because they were below the critical size. Depner and Harwood (1966) suggested that the narrow guts of diapausing *Anopheles freeborni* might be the cause of their reluctance to take blood, but this factor alone is not sufficient to explain the absence of digestion, because gonotrophic dissociation is known in *An. freeborni*, (Washino, 1970).

Briegel and Lea (1975) found that the rate of protein digestion in *Aedes aegypti* was related to the amount of protein ingested, from 4.0 down to 0.5 mg of blood, below the critical meal size of 1.0 mg found by Spielman and Wong (1974). Briegel and Lea suggest that a secretogogue mechanism, without neural involvement, controls blood digestion. In diapausing *Cs. inornata* no trypsin activity was induced in spite of the fact that small amounts of blood were present in their midguts up to 48 hours after feeding, the time of peak trypsin activity in non-diapausing females. Thus the failure of the diapausing *Cs. inornata* to produce trypsin was not due to the small size of the meals, or the short time they were retained in the midgut, and the secretogogue mechanism must have been either repressed or not activated.

What, then, is the most likely activating (or repressing) stimulus for the secretogogue mechanism? Several studies on different groups of insects have shown that juvenile hormone (JH), produced by the corpora allata, is required for development of the ovaries, and diapause occurs when JH is absent, (review by de Wilde and de Loof, 1973). *Culex pipiens* females reared under short days, or reared under long days and allatectomized, had diapause-sized follicles, but topical application of JH to such females resulted in follicle growth, (Spielman, 1974). The corpora allata of diapausing *Anopheles maculipennis messeae* are much larger and differ in staining properties than those of gonoactive females, and appear empty of secretion. After the first blood meal at the end of diapause, the glands shrink and acquire the same staining properties as in gonoactive females, (Detinova, 1945). In the Colorado potato beetle, *Leptinotarsa decemlineata*, allatectomy or cauterisation of the pars intercerebralis releases the entire "diapause syndrome", including cessation of feeding, burrowing, degeneration of the flight muscles, cessation of oogenesis, the appearance of a diapause protein in the haemolymph, and reduced oxygen consumption; implantation of active corpora allata or topical application of JH terminates diapause, (de Wilde and de Loof, 1973). In the blowfly *Calliphora erythrocephala*, the corpora allata increase in volume before and decrease during vitellogenesis in females fed protein and sugar ad lib, and remain small in females fed sugar only. The blowflies feed preferentially on protein prior to vitellogenesis and preferentially on carbohydrate after vitellogenesis has begun. This shift to carbohydrate does not occur in allatectomised females, (Strangways-Dixon, 1961, 1962). Since diapausing mosquitoes feed on sugars it seems likely that the absence of a shift to sugar feeding in allatectomised *C. erythrocephala* was an indirect result of the failure of vitellogenesis, and not the absence of a direct stimulus of JH on sugar feeding.

The simplest explanation for the diapause syndrome in *Cs. inornata* is that JH not only promotes follicle development but also stimulates blood feeding and activates the secretogogue mechanism of protein digestion. Unfortunately this explanation will not accomodate the phenomenon of gonotrophic dissociation, since one could not have blood feeding and digestion without ovarian development. Thomsen and Moller (1959) reported that in *Calliphora erythrocephala* removal of the median neurosecretory cells resulted in failure to produce proteinases after meat feeding, though they did not demonstrate that the meal was transported to the digestive part of the gut. It does suggest, however, that the secretogogue mechanism may require neurosecretory activation. A modified theory, fitting the information I have summarised, would be as follows:

a) A brain hormone, the production of which is affected by daylengths controls blood feeding and protein digestion directly, and follicle development indirectly, via the corpora allata.

b) In gonotrophic dissociation the neurosecretory system continues to promote blood feeding and digestion, but the corpora allata do not respond.

c) In diapausing females such as *Cs. inornata*, the neurosecretory system is inactive.

In *Cs. inornata* a break must occur early in the chain of events leading to blood-feeding since diapausing females fed after being placed within a few cm of my arm. Diapausing females might also feed if they came close to a vertebrate in nature. This might occur in the depths of a burrow, and even though digestion of the blood and egg development would not occur, 48 hours should be long enough for viruses to penetrate the midgut. Such females would remain nulliparous.

9.1.7. Laboratory induction of diapause

Diapause in *Cs. inornata* was induced by a decrease in daylength from 16 to 12 hr at the IVth instar or at pupation, but not by constant daylengths of 16 or 12 hr, (Chapter 6). The diapausing females that appeared in nature en masse in mid-August would have experienced a decrease in daylength from approximately 18 hr at hatching to 16 hr at emergence, (Chapter 5). The critical daylength for diapause induction is therefore uncertain, but there must be one, since not all females developing after the summer solstice go into diapause. Other examples of the long day/short day response are known in other insects, (see Section 6.5.), and further studies will probably reveal this response in other mosquitoes.

The duration and intensity of diapause was not studied in laboratory-reared females. Most wild-caught *Cs. inornata* were aroused from diapause after 7 days at 16 hr/20 C in one experiment, but not in another, and a few that survived three months at 12 hr/2 C seemed to have terminated diapause, (Section 4.3.4.). *An. earlei* in a dark root cellar at a mean temperature of 6.4 C had terminated diapause by mid-January, (Section 4.3.1.). Tauber and Tauber (1976) provide a system of classification, with examples, of the ways in which daylength or changes in daylength may maintain or terminate diapause. *Cs. inornata* and *An. earlei* cannot be placed in the system with the available evidence. They do not, however, resemble the all-or-none type of response in the mosquito *Wyeomyia smithii*, in which larval diapause could be broken rapidly at any time in the winter by transfer to constant daylengths exceeding 14.5 - 15 hr, (Smith and Brust, 1971).

9.1.8. Cold-hardiness

An. earlei and *Clx. territans* survived 5 1/2 months in winter at temperatures below 0 C. During most of the winter at most sites they were probably supercooled, because their supercooling points were around -20 C, while the temperatures in the overwintering sites rarely went below -10 C. A low of -31 C was recorded in one of the rockpiles, however, and live *An. earlei* and *Clx. territans* recovered after this low from an adjacent rockpile, with similar snow cover, had mean supercooling points higher than this. This suggests that they must have survived freezing. Freezing in the laboratory to determine supercooling points was fatal to all species examined, but this may have been a result of the relatively rapid cooling.

Cs. inornata females were not found in winter, but their much higher supercooling points and poor survival at temperatures below 0 C suggest they could overwinter only in well-insulated sites, such as the depths of burrows. McLean et al (1975) report survival of *Cs. inornata* females from Marsh Lake, Yukon, for 117 days at -1 C. This cold-hardiness and the fact that they were apparently able to get large numbers of females at human bait suggest that they may have been dealing with another species of *Culiseta*, possibly *Cs. impatiens.*

9.2. Conclusions

From the evidence presented it is difficult to find a role for any of the mosquito species studied in the ecology of Western Encephalitis (WE). The ornithophilic *Culiseta morsitans dyari* seems the most likely to be involved in enzootic transmission, and further study of this species may prove fruitful. Since *Cs. m. dyari* overwinters in the egg stage, transovarial transmission is a possibility. My results for *Cs. inornata* do not explain how they become infected with WE in central Saskatchewan, (McLintock et al, 1970).

Since all but one of the overwintering females were nulliparous, and the females did not take blood in the fall, it is most unlikely that they overwinter WE, or any other disease- causing organisms. This does not rule out the possibility of transmission in early spring, when *Cs. alaskaensis* and *An. earlei* feed on a wider range of hosts than they do later in the year.

Cs. inornata seems well-adapted to Central Alberta in some ways but not in others. The preference of the females for feeding on cattle, and their wide powers of dispersal (Clarke, 1943) may be the factors responsible for the high populations observed. On the other hand, the poor cold-tolerance and meagre food reserves of females in the fall suggest that they would be restricted to overwintering sites that were not cold enough to freeze them or warm enough to starve them, probably at around 0 C. Such sites may be scarce, and overwintering in the region is probably achieved at the cost of very high mortality. Dispersal in spring from these few foci would depend on the prevailing winds and would explain the rather erratic appearance of the females at any given site.

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APPENDIX

Records of air and water temperatures
at George Lake,
1973 - 1975

Table 73. Air temperature records (C) at George Lake, May to October, 1973-75. Records from thermohygrograph in Stevenson Screen, in aspen woods near lake shore.

Month and decade		1973						1974						1975					
		Days (a)	Mean max	Mean min	Mean mean (b)	Abs max	Abs min	Days	Mean max	Mean min	Mean mean	Abs max	Abs min	Days	Mean max	Mean min	Mean mean	Abs max	Abs min
May	I		12	2	7	19	-1	0	-	-	-	-	-		15	2	8	21	0
	II		22	6	14	29	5		14	2	8	19	-1		17	3	10	21	2
	III	0	-	-	-	-	-		18	3	10	25	0		16	2	9	21	-1
June	I	4	17	3	10	25	0		18	7	12	22	4		20	5	12	26	2
	II	5	16	7	12	21	4		25	9	17	29	7		18	7	12	22	5
	III	8	21	8	14	26	3		22	10	16	29	5		19	8	13	26	5
July	I		21	9	15	26	7		20	9	14	25	7	7	27	12	19	29	8
	II		22	9	15	28	4		22	11	16	28	10	5	18	11	14	21	10
	III		22	11	17	28	7		23	10	17	26	7		19	10	14	26	7
Aug	I		21	14	18	27	12		23	9	16	29	7		19	8	14	22	5
	II	7	21	10	15	26	8		18	7	12	25	4		16	8	12	21	2
	III		17	9	13	22	5		18	8	13	20	5		16	8	12	22	5
Sept	I		19	8	13	26	2		12	3	7	25	0		15	4	9	21	0
	II		10	0	5	16	-3		20	6	13	26	0		18	3	10	24	0
	III	7	9	5	7	16	3	6	9	2	5	21	-5		19	4	12	26	2
Oct	I		7	-2	2	14	-3		14	1	8	27	-1	0	-	-	-	-	-
	II		9	0	4	12	-8		17	2	9	22	0	0	-	-	-	-	-
	III		5	-2	2	9	-6		13	-1	5	19	-2	0	-	-	-	-	-

(a) Days recordings - 10 or 11 per decade, except where otherwise indicated.

(b) $\text{Mean mean} = \dfrac{\text{Mean maximum} + \text{Mean minimum}}{2}$

Table 74. Water temperature records (C) in lakeside pond, George Lake, May to October, 1973-75. Records from bourdon tube recorder, in shade of willow tree.

Year	1973				1974				1975				1975			
Depth(cm): pond	20				55-75				50-80				50-80			
probe	10				2.5				2.5				30-60(c)			
Month and decade	Days (a)	Mean max	Mean min	Mean mean (b)	Days	Mean max	Mean min	Mean mean	Days	Mean max	Mean min	Mean mean	Days	Mean max	Mean min	Mean mean
May I	0	-	-	-	0	-	-	-		11	5	8	0	-	-	-
II		21	8	14	0	-	-	-		17	10	13	0	-	-	-
III		17	10	13	8	19	13	16		17	10	13	0	-	-	-
June I		19	8	14		18	13	16		19	13	16		12	12	12
II		17	9	13		22	16	19		13	13	13		12	12	12
III		20	14	17		21	16	18		18	13	16		12	10	11
July I		17	13	15		19	14	17		24	17	21		13	12	12
II		19	12	15	5	22	18	20		19	16	17		14	14	14
III	7	18	12	15		20	16	18		18	14	16		13	12	12
Aug I	0	-	-	-		18	15	16		16	12	14		12	12	12
II	4	10	8	9		14	11	13		13	10	11		10	9	9
III		12	10	11		14	10	12		13	10	12		10	10	10
Sept I		14	9	12		11	8	9		13	9	11		9	9	9
II		9	6	7		11	8	9		13	8	10		9	8	8
III		8	6	7		9	5	7		13	8	10		8	8	8
Oct I		6	2	4		6	2	4	0	-	-	-	0	-	-	-
II		4	2	3	9	6	3	4	0	-	-	-	0	-	-	-
III		4	2	3	0	-	-	-	0	-	-	-	0	-	-	-

(a) Days recordings = 10 or 11 per decade, except where otherwise indicated.

(b) Mean mean = $\frac{\text{Mean maximum + Mean minimum}}{2}$

(c) At bottom of free water, lying on 20-30 cm of loose debris.

Table 75. Day-degrees above 5 C in air and lakeside pond, George Lake, July and August, 1974-75. From records gathered as described in tables 73 and 74.

Day	July				August			
	Air		Water		Air		Water	
	1974	1975	1974	1975	1974	1975	1974	1975
1	8	11	11	12	10	10	11	10
2	9	14	12	15	12	9	12	9
3	10	16	12	18	15	18	14	9
4	11	17	13	18	15	8	14	9
5	10	17	12	18	13	9	13	10
6	8	17	12	17	9	8	12	9
7	8	11	11	15	8	10	9	10
8	10	12	11	14	9	7	9	8
9	11(s)	13	12	15	10	7	10	8
10	12(s)	15	14	15	10	8	10	8
11	9(s)	17	12	16	8	8	8	8
12	8(s)	18	10(E)	17	5	5	7	8
13	10(s)	13	12(E)	15	6	8	6	7
14	11(s)	14	14(E)	14	8	7	8	8
15	15(s)	11	17(E)	13	8	5	8	7
16	11	11	15	11	10	5	8	6
17	13	10	16	11	11	6	8	5
18	15	8	17	10	12	5	9	5
19	14	11	16	11	3	5	8	6
20	11	11	13	11	6	9	6	7
21	11	7	13	10	9	9	7	8
22	13	10	14	10	9	7	8	7
23	13	11	15	11	9	7	8	6
24	13	8	14	12	7	8	7	6
25	9	11	12	12	6	3	6	5
26	9	12	12	12	7	6	6	5
27	9	15	12	13	9	9	8	7
28	11	10	12	12	8	11	8	9
29	14	10	13	10	10	6	9	7
30	13	7	13	9	7	5	8	5
31	11	9	11	9	7	5	8	5
Monthly totals	340	377	393	402	276	223	273	227

s = Record for Sion, 3.6 Km S. George Lake. E = Estimate.